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Sensitivity of Vertebrate Embryos to Boron Compounds

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16. ABSTRACT

Developmental stages of rainbow trout, channel catfish, goldfish, leopard frog, and Fowler's toad were treated in a continuous flow system with boric acid and borax concentrations ranging from 0.001-300 ppm boron. Exposure was initiated subsequent to fertilization and maintained through 4 days posthatching, using water with hardness levels of 50 and 200 ppm $CaCO_3$. Expressed in ppm boron at 4 days post-hatching, LC_1 values for trout, catfish, and goldfish were 0.1, 0.5, and 0.6 for boric acid in soft water, 0.001, 0.2, and 0.2 for boric acid in hard water, 0.07, 5.5, and 1.4 for borax in soft water, and 0.07, 1.7, and 0.9 for borax in hard water. LC_{50} values in ppm for trout, catfish, and goldfish were 100, 155, and 46 for boric acid in soft water, 79, 22, and 75 for boric acid in hard water, 27, 155, and 65 for borax in soft water, and 54, 71, and 59 for borax in hard water. The LC_{50} values for amphibian embryos and larvae ranged from 47 for borax in soft water to 145 for boric acid in soft water. By comparison, boron LC_{50} values for chick embryos, treated by yolk injection, were 1.0 and 0.5 ppm for boric acid and borax, respectively.

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FINAL REPORT

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Final Report

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INTRODUCTION

Boron in natural ground and surface waters of the United States usually does not exceed 1 mg/l, and generally is under 0.1 mg/l (1,2). Out of 84 major basins and water supplies recently sampled, only 6 contained boron in excess of 1 mg/l (3). In streams and other water resources of the southeastern U.S., Boyd and Walley (4) reported boron levels under 0.1 mg/l for 86% of 199 samples taken. Considering 1546 samples of river and lake waters from various parts of the United States, Kopp and Kroner (5, 6) reported a mean boron concentration of 0.1 mg/l, with a maximum of 5.0 mg/l. However, in certain western areas of the U.S., boron concentrations of 5-15 mg/l have been reported (1). Recent projections, based largely on dissipative commercial uses of boron-containing compounds, forecast detectable increases in the boron contamination of surface and ground waters. Based on 1972 production figures, it has been estimated that 32,000 metric tons of boron contaminants enter the U.S. environment yearly (7). In a report prepared for EPA by Versar, Inc., the principal sources include laundry products, certain agricultural chemicals and fertilizers, mining and processing of borax, manufacture of glass and ceramics, and coal combustion. Boric acid and borates (e.g., borax) are the major boron-containing pollutants affecting water resources (2, 7-9).

The most critical biological effect of boron so far reported concerns certain plants. Though boron is an essential element in plant nutrition, the growth of more sensitive plants (e.g., cherry, elm, grape, lemon, peach) is inhibited by concentrations exceeding 0.3-1.0 ppm, depending on the species (1, 2, 8, 10).

With respect to animals, bioassay experiments with several species of adult fish have defined lethal dosages which are well above the boron levels generally found in natural water resources (8). Working with the mosquito fish (*Gambusia affinis*), Wallen, *et al.* (11) reported 96-hr TL_m values of 3600 and 5600 mg/l for sodium borate and boric acid, respectively. Using the figure given for boric acid, this amounts to an approximate dosage of 980 ppm in boron equivalents. Turnbull, *et al.* (12) determined a 24-hr TL_m of 15,000 mg/l for bluegill treated with boron trifluoride administered at 20° C in Philadelphia tap water, and a 48-hr LD_{50} of 339 ppm boron has been reported for 15-month old rainbow trout (2, 13). In addition, Sprague (2) has reported safe limits of 30 ppm and 33 ppm for largemouth bass and bluegill, respectively.

Relatively few studies have been conducted on the toxic effects of boron compounds upon developmental stages. However, boric acid has been found to produce high frequencies of lethality and teratogenesis in embryos of several vertebrate species. Takeuchi (15) has shown that limited exposure to a 1% concentration of boric acid is highly toxic to early embryonic stages of the toad (Bufo vulgaris formosus). When treatment was initiated at the yolk plug stage and maintained for 24 hours, only 18% of the embryos survived to reach the external gill stage. Anatomical abnormalities occurred among survivors at a frequency of approximately 42%. The principal anomalies included defects of the notochord, brain, and special sense organs.

Boric acid also has been found to induce mortality and anomalous development in chick embryos (16-19). Landauer (18) treated White Leghorn chicken eggs with 2.5 mg doses of boric acid injected into the yolk at 0-120 hours of incubation. When treatment was initiated at the start of incubation, hatchability was approximately 18% and 85% in experimental and control populations, respectively. Treatment at 96 hours of incubation resulted in 19% hatchability of experimental eggs, and approximately 76% of the embryos which survived through 13 days of incubation were anomalous. In a later study, Landauer (19) performed similar experiments with several strains of the domestic fowl. Using the same dosage, he obtained 0.8%, 12.8%, 15.5%, and 19.6% hatchability for Silver Gray Dorking, White Leghorn, Black Minorca, and White Minorca strains, respectively. Control hatchability was 90% or better for all strains except the Silver Gray Dorking (67%). Following the treatment of eggs with boric acid, the frequency of anomalous chicks in surviving populations varied from 72% for White Leghorns to 96% for the Silver Gray Dorking strain. Teratogenic defects largely affected the skeletal and nervous systems, including rumplessness, anomalous legs and feet, abnormal beaks, cleft palate, various degrees of facial coloboma, reduced or defective eyes, and cyclopia (18, 19). Unfortunately, Landauer did not base the dosage of boric acid on egg yolk volume. However, if we assume a mean value of 18.5 grams for egg yolk, the 2.5 mg dosage used would give an egg yolk concentration of 135 ppm boric acid, or approximately 24 ppm boron. Laying hens were found to tolerate a daily ration containing 0.5% boric acid (875 ppm boron equivalents), though egg production ceased after 6 days of treatment. However, normal egg production resumed within 2 weeks after treatment was discontinued (20).

In addition, Kanakhia, et al. (21) recently noted that boric acid was highly toxic to mammalian embryos. A concentration of 10 ppm boric acid (1.75 ppm boron) prevented 51% of in vitro cultured mouse embryos from reaching the blastocyst stage. By comparison, adult rats were unaffected when maintained for 90 days on rations containing 525 ppm boron, administered either as 3000 ppm boric acid or 4630 ppm borax, and 350 ppm dietary boron produced no adverse effects over a two-year feeding period (2, 22).

Though the studies reviewed above are limited in scope, they provided evidence that embryonic stages are more susceptible to boron contaminants than are adult vertebrates. The principal objective of this study was to determine more precisely the concentrations at which borax and boric acid produce lethality and/or teratogenesis in representative piscine, amphibian, and avian embryos and early posthatched stages.

EXPERIMENTAL METHODS

Selection and source of test animals. Species selected for study included the domestic fowl (Gallus domesticus), leopard frog (Rana pipiens), Fowler's toad (Bufo fowleri), rainbow trout (Salmo gairdneri), channel catfish (Ictalurus punctatus), and the goldfish (Carassius auratus). This selection of animals includes species representing poikilothermy and homeothermy, holoblastic and meroblastic cleavage, two distinct patterns of gastrulation, and a number of other important developmental variables (e.g., egg type, yolk quantity, hatching time) which may respond differentially to boron exposure (24).

Gravid fish and amphibians were obtained from State and Federal hatcheries at Frankfort, Kentucky, Erwin, Tennessee, and Senecaville, Ohio, and from selected sites within Kentucky. Fertile chicken eggs (White Leghorn strain) were obtained from the Poultry Science Department, University of Kentucky.

Test concentrations and exposure periods. Test animals were exposed to 10-14 concentrations of boric acid and borax. Depending on the sensitivity of the animal species, tests were initiated at 50-300 ppm boron and continued at 2 to 10-fold dilutions until LC_1 and LC_{50} values were determined. In all cases, exposure levels of boric acid and borax were based on actual boron content (boron equivalents), and verified by the chemical analysis of culture water.

For aquatic species, boron treatment was initiated subsequent to fertilization and maintained continuously through 4 days posthatching, giving

exposure periods in days of 28, 9, and 7 for trout, catfish, and goldfish, and 7.5 for amphibians. Chicken eggs were treated with boron compounds immediately prior to the onset of incubation, and test responses were ascertained upon completion of the hatching process. Mean hatching time was 21 days.

Sample size and expression of data. Minimum sample size per test concentration was set at 100 for the chick embryo and 125 for frog and fish embryos. In the analyses of test results, embryos were classified as lethals, teratogenic (anomalous) survivors, or normal survivors. Hatchability was based on all embryos which lived to complete the hatching process. Normal survivors were defined as animals free of the debilitating morphological defects which characterized teratogenic survivors (e.g., Plate 1). Test responses were expressed as frequencies in experimental populations/frequencies in controls. Survival of control populations is summarized in Table 23.

Log probit analysis was used to statistically determine LC_1 and LC_{50} values (25). The probit regressions were performed with an IBM computer (model 370-165), and were determined in each instance by using animal responses (e.g., lethality) for the full range of test concentrations. When severely truncated survival curves precluded use of the computer program for LC_{50} determinations, the Litchfield-Wilcoxon graphic method was used (35). Analysis of variance and the t-test were used to determine statistical significance of differences between boric acid and borax toxicity, water hardness levels and other test variables.

Culture water for aquatic embryos. We have given considerable attention to the development of a synthetic culture water suitable for bioassays. Synthetic water provides more stable test conditions than natural water, as the latter is subject to substantial seasonal fluctuations in composition (e.g., total dissolved solids, hardness, pH). Also, problems with background contaminants are minimized when prepared water is used for culture purposes. However, it is essential to use a formulation for synthetic water which gives chemical and physical characteristics similar to natural water.

The culture water described below has been used extensively during the past 2 years, and is known to give toxicity responses with metals (e.g., Cd, Cu, Hg, Zn) which are closely comparable to results obtained using natural water of similar composition. Also, we have performed hatchability tests with 4 species of fish and 5 species of amphibians, comparing our synthetic culture

water to natural water from Steele's Run, a local stream (limestone bed) which supports a healthy aquatic ecosystem. Frequencies of hatchability were statistically indistinguishable for the two water sources, averaging 92% for fish and 96% for amphibians (41).

The synthetic culture medium used for the boron bioassays was prepared from distilled, double deionized water, having a conductivity of 0.25 umhos or less. Routine monitoring was conducted for background contaminants, including mercury, cadmium, lead, and other trace metals. Reagent grade calcium, magnesium, sodium and potassium salts were added as given in Table 1. This basic stock was prepared to give a water hardness level of 200 ppm (as $CaCO_3$) and a pH of 7.5-8.0. Different levels of hardness were achieved by dilution of the basic culture water.

Our basic stock solution has essentially the same pH, conductivity, and water hardness as given for Mount's test water (23), which consisted of a mixture of natural spring water (emerging from limestone strata) and carbo- filtered, deionized tap water. Also, the content of calcium, magnesium and potassium are in close agreement. The concentrations of cations and anions listed in Table 1 fall within ranges published for freshwater resources in Arizona (26), Kentucky (27), and other areas of the U.S. (8). Total chloride content, total dissolved solids, and the concentration of sodium plus potassium are well under maximum levels of 170 mg/l, 400 mg/l, and 85 mg/l, observed in 95% of U.S. waters found to support good, mixed fish fauna (28). Also, specific conductivity of 300 umhos falls within the range of 150-500 recommended for fish propagation (8), and is typical of values recorded for freshwater resources in Kentucky (27). In addition, osmolarity is well within the maximum limit of 50 mOsm/Kg water suggested for freshwaters in the U.S. (1). Total alkalinity exceeds the minimum recommended level of 20 mg/l (as $CaCO_3$), and closely approaches the range cited as optimum to support a diversified aquatic life (1, 8). As maintained in the culture system described below, (continuous aeration), the dissolved oxygen level is approximately 10 mg/l at the culture temperature used for trout embryos (13^{o} C). A minimum of 7 mg/l has been recommended for trout and salmon spawning waters (1). The pH of our culture medium also is well within the range found in natural waters. Bass Becking, et al. (29) observed a pH range of 6 to 8 for 85% of 347 freshwater rivers and lakes surveyed, and pH values of 6.5-8.5 generally are found in the more productive freshwater resources in the U.S. (1).

TABLE 1

SYNTHETIC CULTURE WATER

DISSOLVED SALTS[1] (mg/l)	
$CaCl_2$	150
$MgSO_4 \cdot 7H_2O$	150
$NaHCO_3$	100
KCl	5
CHEMICAL COMPOSITION (mg/l)	
Ca	54.2
Mg	14.8
Na	27.4
K	2.6
Cl	98.2
HCO_3	72.6
SO_4	58.5
Total dissolved solids	328.3
PHYSICOCHEMICAL CHARACTERISTICS[2]	
Hardness (as mg/l $CaCO_3$)	200.0
Total alkalinity (as mg/l $CaCO_3$)	82.0
Conductivity (μmhos/cm)	300.0
Osmolarity (mOsm/Kg H_2O)	12.0
pH	7.9

[1]Prepared in distilled, deionized water (specific conductivity of 0.2 μmhos).

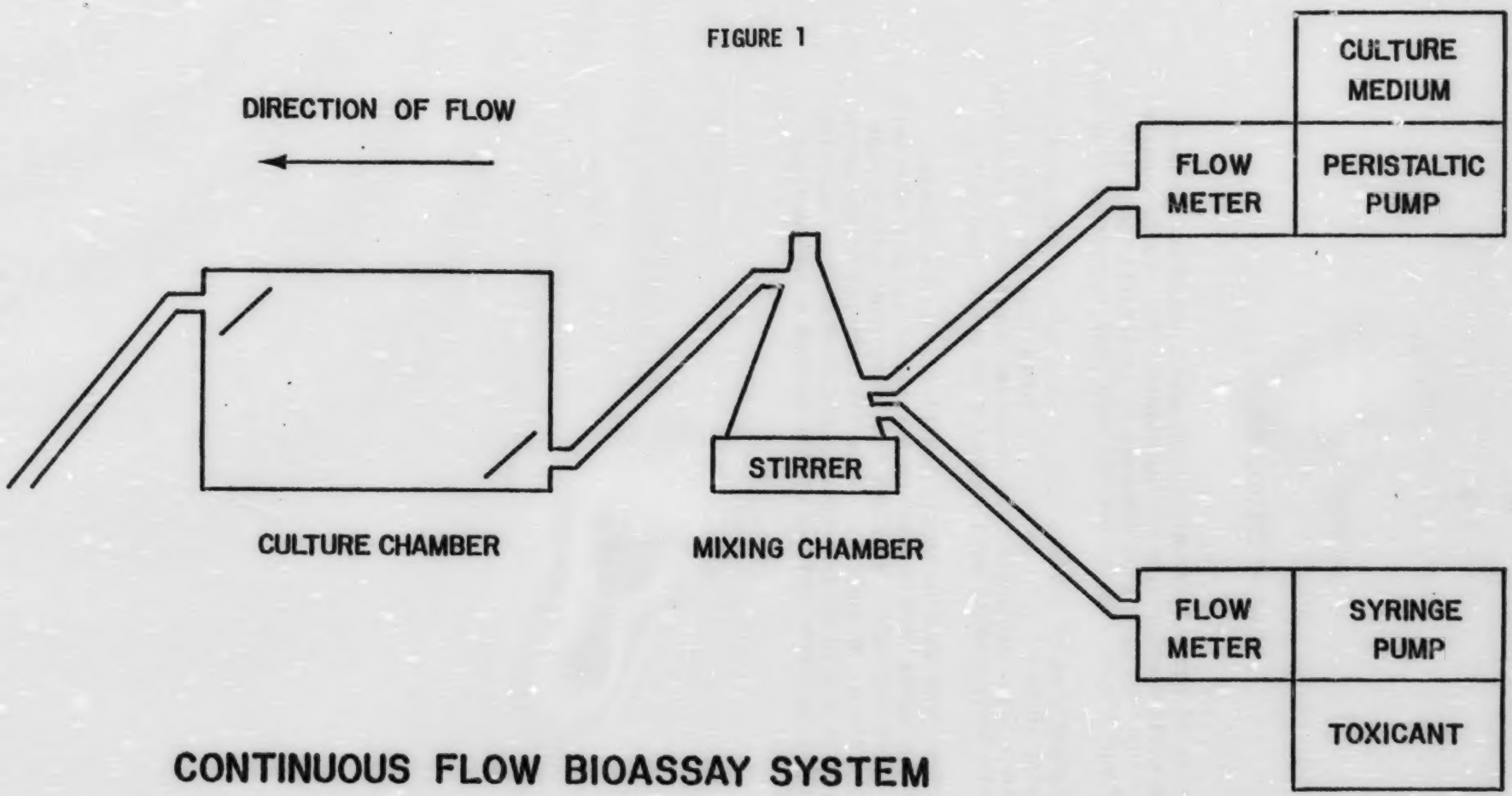
[2]Measurements made at 22° C. Above figures represent mean values for six measurements.

Treatment of aquatic embryos. Boron bioassays were conducted using the continuous flow system described in Figures 1 and 2. Fish and amphibian eggs were cultured through 4 days posthatching in Pyrex chambers, through which test water was perfused at prescribed flow rates. The toxicant was administered to a mixing chamber situated ahead of each culture dish, using graduated flow from a syringe pump. Synthetic culture water was delivered to the mixing chamber by regulated flow from a peristaltic pump. Flow rates from both syringe and peristaltic pumps were monitored by liquid flow meters. Flow rate was set at 200 ml/hr for 300 ml test chambers. Syringe and peristaltic pumps were provided with calibrated, continuously variable speed controls. The concentration of toxicant delivered to the mixing chamber was regulated by adjusting the mixing ratio from the pumping units and/or by varying the concentration of toxicant delivered from the syringe pump. For example (Table 28), to obtain a treatment level of 1 ppb (ug/l), boron at 600 ug/l was delivered from the syringe pump to the mixing chamber at 333 ul/hr, and diluted 1:600 by culture water metered through the peristaltic pump at 200 ml/hr. Solutions from the two channels were mixed with a Teflon-coated magnetic stirring bar, and delivered to the culture chamber under positive pressure.

The system was operated using Brinkmann (model 131900) and Gilson (model HP8) multichannel peristaltic pumps and Sage syringe pumps (model 355). The latter was fitted with a modified syringe holder, and operated using 10 ml double-ground, glass syringes. Syringes were selected for equal stroke volume, and peristaltic pump channels were fitted with tubing of matched diameters.

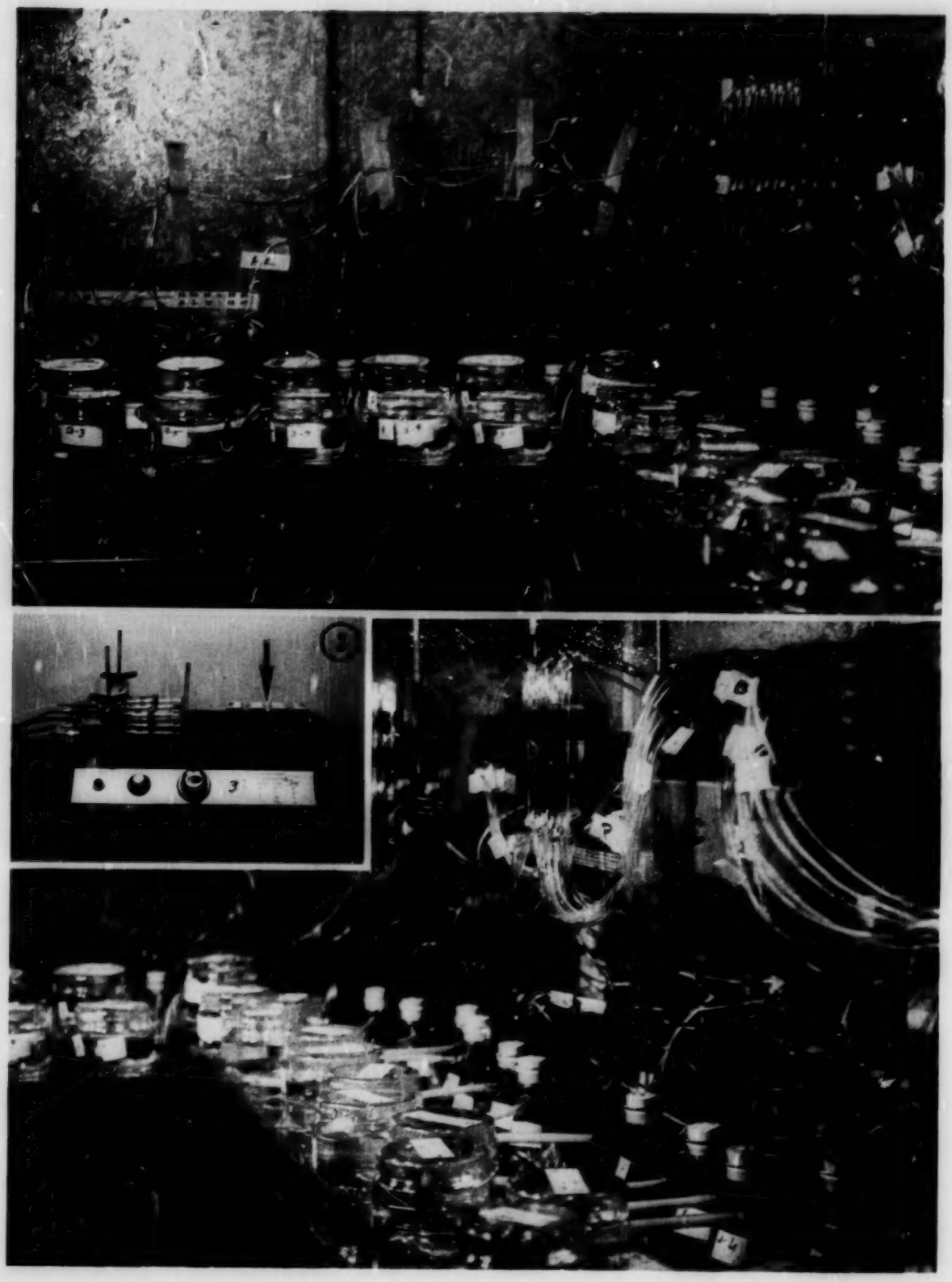
Initially, methylene blue dye was used to check the accuracy of a 1:1000 syringe/peristaltic pump mixing ratio, and to allow visual inspection of mixing and flow patterns through the culture system. Results are summarized in Table 2. Reproducibility of flow rates from syringe and peristaltic pumps is treated in Table 3. The accuracy obtained in regulating boron toxicants is shown in Tables 5-22. For test concentrations of 10 ppm or more, boron compounds generally were added directly to the culture water in the peristaltic pump reservoir, eliminating the need for the syringe pump channel. In such instances, the toxicants remained stable at selected test concentrations for 24 hours or more in polyethylene containers, and important test parameters of culture water were not altered upon standing (e.g., pH, hardness).

FIGURE 1



CONTINUOUS FLOW BIOASSAY SYSTEM

Peristaltic pump delivers culture water to mixing chamber at a prescribed flow rate. Toxicant is administered to the mixing chamber by graduated flow from a syringe pump, providing toxicant/culture water mixing ratios down to 1:1000 or less. The final mixture, agitated with a teflon-coated stirring bar, is pumped through the egg culture chamber under positive pressure. Mixing baffles in the culture chamber facilitate uniform flow. High resolution liquid flow meters are used to determine flow rates from syringe and peristaltic pumps.



All aquatic bioassay cultures were maintained in walk-in environmental rooms. Culture water was given continuous aeration (filtered air) in the peristaltic pump reservoirs. For the 18 bioassays, pH ranged from 7.5-7.9 and 7.8-8.5 for boric acid and borax, respectively (Table 4). All cultures were monitored at regular daily intervals for temperature, dissolved oxygen, ammonia, water hardness and pH, using a YSI tele-thermometer with thermocouple, YSI oxygen meter (model 51A), Orion ammonia and water hardness electrodes, and a Corning digital pH meter (model 110, with expanded millivolt scale). Flow rates from peristaltic and syringe pumps were monitored twice daily. The concentration of ammonia was held below 0.1 ppm in all instances, and other test parameters were maintained as given in Table 4.

Boron exposure levels were determined at regular daily intervals by analysis of culture water. Cultures were maintained at temperatures of 13-14^{o}C for trout embryos, 24.7-29.4^{o}C for catfish, and 24.8-27.0^{o}C for goldfish, giving average hatching periods of 24, 5, and 3 days, respectively (Table 4). Hatching time averaged 3.5 days for Fowler's toad (24^{o}C) and the leopard frog (25^{o}C). Test organisms were examined daily to gauge extent and frequency of development, and to remove dead specimens. Control eggs were cultured simultaneously with experimentals and under identical conditions, except for omission of the toxicant.

Particular attention was given to trout embryos, especially during the "green stage". Harmful exposure to artificial light was precluded (30, 31) and cultures were maintained under semi-sterile conditions to minimize occurrences of soft egg disease and fungus. Prior to each use, culture rooms were disinfected and irradiated with UV for 12 hours, and the rooms were maintained under positive pressure.

In bioassays with boron toxicants, we maintained a flow rate of 200 ml/hr, giving a turnover time of 1.5 hours. As shown in Table 33, we have not found flow rate to constitute a substantial test variable in bioassays with certain metallic toxicants (e.g., Cd), providing the turnover is sufficiently high to prevent deterioration of culture water.

Trout eggs and sperm were collected for test purposes by artificial spawning and milking, using methods of Leitritz (32). Fertilization was accomplished by mixing sperm and eggs for 15 minutes immediately prior to the onset of boron exposure. For all other aquatic species, fertilized eggs were collected

from natural spawn. Boron treatment was initiated immediately after spawning for amphibian species and up to 2 hours post-spawning for goldfish and catfish.

Treatment of chick embryos. Chicken eggs were treated by yolk injection, administering the selected boron compound in a 0.1 ml aliquot of sterile, distilled water at a concentration calculated to dilute yolk volume to the desired test level. The test solution was deposited in a "needle track" extending through the diameter of the egg yolk.

Eggs were treated just prior to the start of incubation, and injections were performed in a sterile transfer chamber, using a 1.0 ml tuberculin syringe equipped with a 1½" hypodermic needle. Prior to injection, the blunt end of the egg was cleansed with an alcohol swab, and a small hole was drilled over the air cell. The needle was inserted into the air cell of the egg and extended through the diameter of the yolk. As the needle was withdrawn, the test aliquot was deposited in the needle track, as previously described by Birge and Just (33). This procedure provides more uniform dispersal of toxicant than do injections which are directed into the central yolk mass. Control eggs were injected in identical manner, except the toxicant was withheld from the aliquot of distilled water. Hatching frequencies of control eggs ranged from 78-89%. Fertility level, determined on independent samples of uninjected eggs, varied from 82-91%. Control and experimental eggs were incubated simultaneously for 21 days at 38°C and a relative humidity of 65%.

Analytical procedures. Quantitative determinations on boron were accomplished using the curcumin method. Following the procedures given in Standard Methods (34), this technique proved applicable for a concentration range of 0.1-1.0 mg/l. Concentration and dilution of sample water were used to extend the analytical range (34). Boron monitoring results obtained are given in Tables 5-22. In addition, all lots of prepared culture water were monitored for possible boron contamination prior to use for bioassay purposes. In no instance was background boron contamination detected in the prepared culture water, including that used for the maintenance of control animals. To avoid possible contamination from borosilicate glassware (42), Vycor brand evaporating dishes were used for analytical purposes.

Actual exposure levels below 0.1 ppm boron were not included in Tables 5-22, as analyses of standards prepared at 0.05 ppm or less were not fully reproducible. However, in all such cases, boron levels delivered by syringe

pumps to mixing chambers were well above the detection limit, and direct analyses were conducted to confirm syringe pump boron concentrations. In addition, flow rates from syringe pumps (boron) and peristaltic pumps (culture water) were monitored at regular intervals, using both flow meters and direct volumetric measurements, to determine actual boron dilution ratios obtained in the mixing chambers. Dividing actual syringe pump boron concentrations by boron dilution ratios, final boron levels delivered to the bioassay test chambers usually were found to be within 3%, and always within 5%, of selected nominal exposure values. In Table 28, this alternative monitoring procedure is shown for trout bioassays, which included boron exposure values of 0.01 ppm (10 ug/l) and 0.001 ppm (1 ug/l). Though such measurements may be used to confirm boron concentrations delivered to test chambers, they do not provide direct measurements on the stability of exposure levels maintained within the bioassay cultures. While it is unlikely that actual boron exposure values could have exceeded the upper limit of variation (5%) shown for water supplied to the cultures, possible losses of boron from culture water, through tissue accumulation or other means, could have resulted in undetectable reductions in exposure concentrations. However, as seen in Tables 5-22, a high degree of culture stability was obtained for boron at concentrations as low as 0.1 ppm, as determined by direct analyses of culture water.

Boron compounds. The boron compounds used for testing were obtained from the Fisher Scientific Company. Both boric acid (H_3BO_3) and borax, sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10\ H_2O$), were certified ACS grade. All results were calculated and presented as ppm boron.

EXPERIMENTAL RESULTS

Bioassays with aquatic embryos. General water quality parameters maintained in the aquatic bioassays are summarized in Table 4, and the resulting toxicity data, together with analyses of actual boron exposure levels, are given in Tables 5-22. In all instances, test responses are control adjusted, as noted under Experimental Methods (i.e., frequencies in experimental populations/ frequencies in corresponding controls). Frequencies of survival and teratogenesis for control populations of aquatic embryos are presented in Table 23. Probit analyses of boron LC_1 and LC_{50} values are given in Tables 24 and 25.

Experimental results are based on continuous flow treatment from fertilization through hatching and 4 days posthatching. Results given at 4 days

posthatching include accumulative test responses recorded for the entire exposure period. Frequencies of hatchability are based on all animals which lived to complete the hatching process, including teratogenic survivors. Percentages of teratogenic animals observed in hatched populations are given parenthetically in Tables 5-22. Their frequencies include only animals found to bear gross, debilitating anomalies, as determined by microscopic and cinematographic observations. Animals free of such defects were treated as normal survivors. For convenience, results are presented using nominal concentrations for exposure levels and water hardness. Actual boron exposure values are given in Tables 5-22 for bioassays with boric acid and borax administered in soft and hard water (50 and 200 ppm $CaCO_3$). Measurements on water hardness are shown in Table 4.

Rainbow trout. Developmental stages of the trout were exposed for 28 days to boron concentrations ranging from 1 ppb to 200 ppm. Average hatching time was 24 days at 13-14°C, and the results are summarized in Tables 5-8. From 1 ppm to 200 ppm boron, percent hatchability of trout eggs generally was inversely proportional to exposure level. At 1 ppm boron, hatchability varied from 94-99%. Borax at or below 0.5 ppm boron did not reduce hatching frequency, compared to control populations. Treatment with low concentrations of boric acid gave more variable results, with 5-8% embryonic lethality occurring at boron concentrations as low as 0.01 ppm (Tables 5, 6). High concentrations of boron were required to produce substantial levels of embryonic mortality. At 200 ppm, complete lethality was observed with borax, and hatching frequencies were reduced to 55% and 65% for boric acid in soft and hard water, respectively. Using 50 ppm boron, egg hatchability varied from 77-82% for boric acid to 60-80% for borax.

Boric acid produced the highest frequencies of teratogenic survivors, particularly when used at a water hardness level of 200 ppm $CaCO_3$. In the latter case, 51% of those animals which survived 100 ppm boron were grossly defective (Plate 1), and frequencies dropped to 26%, 11%, and 5% at 1.0, 0.01, and 0.001 ppm boron, respectively. It is especially noteworthy that a high incidence of teratogenesis was observed over a broad range of exposure levels, varying from 200 ppm to 1.0 ppm boron (Table 6). Results were much more variable and less pronounced when boric acid was administered in soft water (50 ppm $CaCO_3$), with 27%, 1%, 21%, and 5% of survivors bearing anomalies at exposure levels of 200, 50, 1.0, and 0.01 ppm boron (Table 5).

Exposure of trout eggs to borax consistently produced 2% to 3% teratogenic survivors among populations exposed to boron concentrations of 0.001 to 10.0 ppm. Frequencies of anomalous survivors exceeding 10% occurred only at 25 ppm boron or more, and water hardness did not appear to constitute a significant variable.

To best determine the effects of boron treatment on survival of test organisms, frequencies of lethality and teratogenesis were combined and the resulting data are expressed in Tables 5-8 (e.g., percent dead or teratogenic). Survival frequencies for animals found to be free of gross teratogenic anomalies also are given (percent normal survival). Though classified as normal, such animals were not screened for possible physiological, genetic or behavioral impairments. Using the combined data, whether expressed as percent normal survival or as percent dead or teratogenic, results were somewhat less proportional to boron exposure level than was percent egg hatchability. This was more evident in studies with boric acid, in which teratogenesis was substantially less dependent on boron concentration than was embryonic mortality (Tables 5, 6).

Probit analyses of the combined data for boric acid at 4 days posthatching gave LC_{50} and LC_1 values of 100 ppm and 0.1 ppm boron in soft water, and 79 ppm and 0.001 ppm boron in hard water (Tables 24, 25). With borax, LC_{50} and LC_1 values at 4 days posthatching were 27 ppm and 0.07 ppm boron in soft water and 54 and 0.07 ppm in hard water. These values reflect the sum total of three test responses, including embryonic mortality, embryonic teratogenesis, and posthatched mortality. At boron concentrations ranging from LC_1 values to 50 ppm, embryonic mortality and teratogenesis were the predominant responses. This is evident in the values for percent normal survival which showed little or no decline from hatching to 4 days posthatching, except at extremely high boron concentrations (Tables 5-8).

Boric acid produced higher rates of embryonic mortality and teratogenesis when administered in hard water (Tables 24, 25). However, water hardness was not an appreciable factor concerning the embryopathic effects of borax. This is especially evident from a comparison of borax LC_1 values (Table 24), as well as results obtained at exposure levels of 0.001 to 10.0 ppm boron (Tables 7, 8). Concerning the 4 trout experiments (Table 24), the order of increasing boron toxicity to embryonic stages included boric acid in soft water (LC_1 = 0.1), borax in hard water or soft water (LC_1 = 0.07), and boric acid in hard water (LC_1 = 0.001).

The best indication of boron sensitivity of the posthatched trout alevins is given by comparing survival at hatching and 4 days posthatching for exposure levels of 50-200 ppm (Tables 5-8). Results indicate that, in contrast to embryonic stages, trout alevins are more susceptible to borax and that toxicity for both boric acid and borax is slightly higher in soft water. For example, borax at 50, 100, and 200 ppm boron gave posthatched survival values of 22%, 0%, and 0% in soft water and 73%, 0%, and 0% in hard water (Tables 7, 8). It is of interest that the survival of posthatched stages was not substantially affected by boron until exposure levels exceeded 25-50 ppm and 100 ppm in bioassays with borax and boric acid, respectively. However, except for boric acid administered in hard water, survival of posthatched stages dropped sharply once these levels were exceeded.

In all four trout bioassays, boron exposure levels spanned a dilution range of 200,000 without achieving the full range of test responses (e.g., 0% to 100% normal survival). The test responses most characteristic of boron toxicity to embryonic and early juvenile stages of the rainbow trout may be summarized as follows: generally, high frequencies of both embryonic and postembryonic mortality were recorded only at boron concentrations of 50 ppm or more. Embryonic mortality and teratogenesis were the principal boron-induced responses at 50 ppm or less. Using borax, embryonic mortality and teratogenesis decreased progressively with concentration to 3-4% at 1 ppm boron, and 2-3% teratogenic impairment occurred over a broad, near-threshold range of 0.5-0.001 ppm. Though results with boric acid were more variable, trout embryos suffered mortality and teratogenesis at frequencies of 10% or more at boron levels as low as 0.01 ppm, and 4-7% of test animals were affected by 0.001 ppm at 4 days posthatching. While water hardness did not exert a profound influence on boron bioassays with trout, hard water generally increased embryonic lethality and teratogenesis, and soft water increased boron toxicity to posthatched alevins.

The fact that high concentrations (25-200 ppm) were required to consistently produce substantial impairment of test populations indicates that boron compounds are not highly toxic to trout embryos and alevins. However, compared to trace metals such as cadmium and mercury (24), borax and boric acid are unusual in that they exert low level embryopathic effects on trout over a wide span of exposure levels (e.g., 0.001-1.0 ppm).

Channel catfish. Developmental and early posthatched stages of the catfish were treated continuously for 9 days with boron compounds. Average hatching

time was 5 days at 25-29°C. Boron exposure levels ranged from 0.01-300 ppm for boric acid bioassays and 0.05-300 ppm in tests with borax. Both toxicants were used in soft and hard water (50 and 200 ppm $CaCO_3$). For all catfish bioassays, percent egg hatchability and percent normal survival at hatching and 4 days posthatching gave good inverse correlations with boron concentration. For example, correlation coefficients for boron exposure level and percent normal survival at hatching were -0.95, -0.93, -0.95, and -0.98 for catfish embryos treated with boric acid at a water hardness of 50 ppm $CaCO_3$, boric acid at 200 ppm $CaCO_3$, borax at 50 ppm $CaCO_3$, and borax at 200 ppm $CaCO_3$, respectively (Tables 9-12). In all four tests, 300 ppm boron produced lethality or teratogenesis at hatching in 100% of the experimental populations, and normal survival at 4 days posthatching was only 0-2% at 200 ppm boron. Using boric acid in soft water, frequencies of normal survival at 4 days posthatching increased to 56%, 75%, 95%, and 99% at concentrations of 150, 10, 1.0, and 0.5 ppm boron. Normal survival was 100% at and below 0.1 ppm. Treating with boric acid in hard water, normal survival values at 4 days posthatching were 9%, 65%, 86%, and 98% at 75, 10, 1.0, and 0.01 ppm boron, respectively.

Exposure to borax in soft water gave normal survival frequencies at 4 days posthatching of 65%, 70%, 94%, and 98% at 150, 50, 10, and 5 ppm. Normal survival was 100% at and below 1.0 ppm boron. In hard water, continuous exposure to borax for the 9-day treatment period resulted in normal survival for 38%, 71%, 90%, and 96% of test populations at 100, 25, 10, and 1.0 ppm boron. At 0.5 ppm or less, survival was not impaired, except for a 1% deflection at 0.1 ppm.

At 4 days posthatching, LC_1 and LC_{50} values for boric acid were 0.5 ppm and 155 ppm boron in soft water, and 0.2 ppm and 22 ppm in hard water (Tables 24, 25). By comparison, LC_1 and LC_{50} values for borax were 5.3 ppm and 155 ppm boron in soft water, and 1.7 and 71 ppm in hard water. Using LC_1 values, the order of increasing toxicity was borax in soft water, borax in hard water, boric acid in soft water, and boric acid in hard water. The survival curve for borax in hard water at hatching is illustrated in Figure 3. Boric acid was moderately more toxic than borax to both catfish embryos and early fry, and both compounds produced greater impairment of test populations when administered in hard water (Tables 9-12). Similar to results with trout, embryonic mortality and teratogenesis increased in hard water, with boric acid producing higher frequencies than borax. However, differences were observed with post-

hatched stages. Catfish fry were somewhat more sensitive to boron in hard water, as seen by comparing differences in frequencies of normal survival at hatching and 4 days posthatching for high exposure levels (Tables 9-12). As in trout bioassays, boron concentrations of 50 ppm or more were required to produce high levels of posthatched mortality. However, catfish fry were somewhat more susceptible than were trout alevins to lower concentrations of boron. For example, in tests with boric acid in hard water, appreciable levels of post-hatched mortality occurred at all concentrations down to 1.0 ppm boron (Table 10).

The boron dilution ranges required to give test responses varying from 0-100% were approximately 300 for borax in soft water, 600 for borax in hard water, 3000 for boric acid in soft water, and 30,000 for boric acid in hard water. It is apparent that the effective dilution range increases with the order of toxicity. As the boron level required to produce 100% mortality was approximately the same in the 4 different bioassays, this increase largely reflects extensions at the LC_1 end of the exposure range, correlating principally with observed embryopathic effects of boron (e.g., mortality, teratogenesis).

Considering posthatched LC_1 values, catfish stages collectively were less sensitive to boron than trout stages by approximate factors of 200, 80, 25, and 5 for boric acid in hard water, borax in soft water, borax in hard water, and boric acid in soft water. Differential sensitivity was greatest under conditions which proved most toxic to both species (i.e., boric acid in hard water).

Goldfish. Developmental stages of the goldfish were treated continuously with boron compounds for 7 days. Average hatching time was 3 days at 25-27°C. As seen in Tables 13-16, hatchability and normal survival gave good inverse correlations with boron concentration. Boron at 200 ppm produced complete lethality in all tests. Treating with boric acid in soft water, frequencies of normal survival at 4 days posthatching were 4%, 52%, 94%, and 98% for boron concentrations of 100, 50, 10, and 1.0 ppm (Table 13). With hard water, normal survival averaged 35%, 67%, 85%, and 98% for the same exposure levels (Table 14). Borax at 50 ppm hardness gave normal posthatched survival at frequencies of 4%, 78%, 91%, and 97% for 100, 50, 10, and 1.0 ppm boron. Survival frequencies for the same exposure levels were 0%, 77%, 93%, and 95% when hard water was used.

Posthatched LC_1 and LC_{50} values for boric acid, given in ppm boron, were 0.6 and 46 for soft water, and 0.2 and 75 for hard water. For borax, they

were 1.4 and 65 in soft water and 0.9 and 59 in hard water. The order of toxicity for LC_1 values was the same as given for the catfish. However, differences among the 4 bioassays were not as pronounced, as LC_1 values were restricted to a narrow range of 0.2-1.4 ppm boron. The LC_{50} values at 4 days posthatching also fell within a narrow range of 46-75 ppm boron, indicating that water hardness and boron source were less significant than for other piscine species.

As for the trout, substantial levels of posthatched mortality were found only at the higher boron concentrations, and posthatched stages were somewhat more susceptible to boric acid in soft water. Unlike the trout and catfish, appreciable frequencies of teratogenesis occurred only at high exposure levels. Frequencies exceeding 10% were observed only at or above 100 ppm boron.

In all instances, normal posthatched survival was 92% or more at and below 7.5 ppm boron, and there was a broad near-threshold range extending to 0.05 ppm boron at which low levels of embryonic mortality and/or teratogenesis consistently were observed. This is illustrated in Figure 4 for boric acid in soft water, and the boron survival curve may be compared to those for cadmium and zinc obtained with the same test system (Figure 5).

The effective dilution ranges were approximately 4000 and 6000 for borax and boric acid, respectively. Using posthatched LC_1 values, goldfish were less sensitive than trout stages by approximate factors of 200, 20, 13, and 6 for boric acid in hard water, borax in soft water, borax in hard water, and boric acid in soft water. As for catfish and trout, the greatest differential in sensitivity occurred under conditions found most toxic to trout and goldfish (i.e., boric acid in hard water).

Amphibian embryos and larvae. Developmental stages of the leopard frog and Fowler's toad were given continuous treatment for 7.5 days. Average hatching time was 3.5 days at 24-25°C. Leopard frog embryos suffered 100% lethality or teratogenesis when treated with borax and boric acid at exposure levels of 200 ppm and 300 ppm boron, respectively (Tables 17-20). Using boric acid in soft water, normal posthatched survival for the leopard frog increased to 87%, 93%, and 99% at boron levels of 100, 10, and 1.0 ppm. In hard water, normal survival values for the same exposure levels were 88%, 97%, and 100%. Borax in soft water gave normal posthatched survival frequencies of 2%, 39%, 93%, and 99% at concentrations of 100, 50, 10, and 1.0 ppm boron. When borax was administered in hard water, normal survival averaged 16%, 52%, 90%, and 99% for the same exposure levels.

Posthatched boron LC_1 and LC_{50} values for the leopard frog treated with boric acid were 13 and 130 ppm for soft water, and 22 and 135 ppm for hard water. In bioassays with borax, they were 5 and 47 for soft water, and 3 and 54 for hard water. As reflected by these values, borax was distinctly more toxic than boric acid, and water hardness was not a significant factor (Tables 26, 27).

In bioassays with embryos and larvae of Fowler's toad, exposure to boric acid produced 100% lethality or teratogenesis at 200 ppm boron (Tables 21, 22). When treatment was maintained in soft water, normal posthatched survival frequencies were 73%, 98%, and 99% at 100, 50, and 10 ppm boron. Using hard water, survival values were 65%, 72%, 93%, and 98% for exposure levels of 100, 50, 10, and 1.0 ppm. Posthatched LC_1 and LC_{50} values were 25 and 145 ppm when soft water was used, and 5 and 123 ppm for hard water (Tables 24, 25).

Amphibian larvae were surprisingly tolerant to boron compounds, as substantial frequencies of mortality seldom occurred between hatching and 4 days posthatching (Tables 17-22). Also, compared to the fish bioassays, hatchability of amphibian eggs was far less affected by boron treatment. In 4 of 6 tests with amphibian eggs, hatchability ranged from 87-91% at 200 ppm boron. Appreciable reductions in hatchability occurred only with borax treatment of leopard frog eggs at exposure levels of 100-300 ppm boron (Tables 19, 20). Though amphibian embryos and larvae survived boron treatment considerably better than piscine developmental stages, teratogenic impairment was substantially greater at high boron concentrations, frequently involving 90-100% of surviving populations. However, frequencies of teratogenesis dropped sharply between 200 and 50 ppm boron, always reaching 10% or less by 25 ppm, and remaining at 1-3% at lower concentrations. As a consequence, there was an exceptionally broad concentration range, which started at 10-100 ppm boron and extended through 0.05 ppm, where normal survival approached or exceeded 90% but remained below 100%. This unique trend is obvious from data presented in Tables 17-22. When frequencies for mortality and teratogenesis were combined, amphibian stages did not appear significantly more tolerant to LC_{50} concentrations of boron than did piscine species (Tables 25, 27). However, they were appreciably more tolerant to LC_1 concentrations of boric acid, particularly in soft water at hatching ($p < 0.0001$) and 4 days posthatching ($p < 0.03$).

Teratogenesis in aquatic embryos. All control and experimental populations were screened for frequencies of teratogenesis by microscopic and cinematographic observations. Representative cultures were maintained under general

observation for 2 weeks following withdrawal of boron treatment. Embryonic anomalies selected for study were grouped in 6 general categories, as given in Table 29.

Dwarfed body involved morphological irregularities and disproportionate size reductions in the trunk and tail. The body generally was significantly reduced in size compared to the head, and linear compressions in the trunk often were evident, especially between the head and dorsal fin. A moderate case is shown in Plate 1, figure 4. Defects of the cranium largely included reduced or truncated upper jaw (Plate 1, figure 5), immobile, amphiarthrotic lower jaw, or cephalic twinning (duplicitas anterior). The latter condition varied from the occurrence of partial, secondary heads (Plate 1, figure 3) to complete twinning of the head and anterior trunk (Plate 1, figure 2). Amphiarthrotic jaws usually were fixed in the open position (Plate 1, figures 1 and 5).

Defects of the vertebral column were far more frequent than other anomalies, constituting the single, most reliable indicator of boron teratogenicity. This was the only teratogenic response found to occur over the full range of aquatic bioassays (Table 29). The more common patterns included acute lordosis, scoliosis, kyphosis, and extreme, rigid coiling of the vertebral column (Plate 1, figures 1, 3, 4, and 5, respectively). In the order named above, frequencies for these different vertebral defects in trout embryos were 17%, 19%, 14%, and 30% for boric acid in hard water, and 36%, 12%, 22%, and 14% for boric acid in soft water, amounting to 80% and 84% of the total disabilities.

Defective fins were treated as gross anomalies only when absent or reduced sufficiently to impair locomotion. The most common impairment of the nervous system included absent or defective eyes. Retinal coloboma, though not classified as a gross anomaly, frequently occurred in trout alevins which suffered acute defects of the skull or vertebral column (Plate 1, figures 1, 5, and 6).

Other boron-induced abnormalities included deformed or edematous yolk sacs in fish embryos, and acute abdominal edema in amphibian larvae. Retarded yolk sac resorption was classified as a teratogenic response only when complicated by morphological defects. Frequencies for the different categories of teratogenesis are summarized for the 18 aquatic bioassays in Table 29.

Chick embryos. Chicken eggs were injected with doses of boric acid and borax calculated to dilute egg yolk volume to boron concentrations of 0.01-50 ppm. Treatment was initiated immediately prior to onset of incubation, and

all frequencies of hatchability were control adjusted as given under Experimental Methods. Boric acid treatment gave hatchability values of 0%, 54%, and 92% at boron exposure levels of 50, 1.0, and 0.01 ppm (Table 31). The frequency of teratogenic chicks surviving treatment ranged from 4% at 0.50 ppm boron to 10% at 10 ppm. Treatment with borax gave hatching frequencies of 0%, 49%, and 95% at 50, 1.0, and 0.01 ppm boron (Table 32). However, frequencies of teratogenesis were higher with borax, ranging from 42% at 25 ppm to 2-3% at 0.10-0.25 ppm.

The patterns of teratogenic development produced by boric acid and borax were comparable. Nearly 91% of the observed embryonic anomalies involved defective feet and/or toes, frequently accompanied with motor imbalance. Defects of the gut, coelom and ventral body wall comprised 5% of boron-induced defects, including abdominal cysts, hernias, eventrations, and unresorbed yolk sacs. The remaining anomalies (4%) involved absent eyes, occipital encephaloceles, defective beaks and facial coloboma. More bizarre conditions (e.g., rumplessness) were not observed among surviving populations, as grossly defective embryos failed to complete the hatching process.

Combining teratogenesis and lethality, the LC_{50} values for boric acid and borax were approximately 1.0 ppm and 0.5 ppm boron, respectively. The LC_1 values for these compounds appeared close to 0.01 ppm boron. No teratogenic development was observed at this exposure level, and hatchability was 92-95% of that observed for controls.

CONCLUSIONS

Environmental toxicity of boron. Depending on water hardness and the boron compound administered, the ranges of LC_1 values at 4 days posthatching were 0.1-0.001 ppm for trout, 0.2-1.4 ppm for the goldfish, and 0.2-5.5 ppm for the catfish. The LC_{50} values ranged from 27-100 ppm for the trout, 46-75 ppm for the goldfish, and 22-155 ppm for the catfish. Amphibian species were more tolerant to boron, particularly at low concentrations, with LC_1 and LC_{50} values at 4 days posthatching of 3-25 ppm and 47-145 ppm, respectively.

Due to the somewhat variable responses obtained at low concentrations and the unusual shape of the boron survival curve, 95% confidence limits for LC_1 values could not always be determined by the probit program. However, the probit LC_1 values were in reasonable agreement with test responses given in

Tables 5-22. For the 18 bioassays, using control adjusted data, measurable impairment of test populations was always observed at and above LC_1 concentrations, both at hatching and 4 days posthatching. In addition, the 36 independently derived LC_1 values correlated with actual concentrations which produced mortality and/or teratogenesis at frequencies of 1-7% above control levels.

Generally, concentrations at 100-300 ppm boron produced complete lethality for all species, and differences in sensitivity were reflected primarily at lower exposure levels. Consequently, variations in boron sensitivity among the 5 species were greater for LC_1 values, and significantly less for LC_{50} levels. On the basis of LC_1 values, the order of decreasing species sensitivity was rainbow trout, goldfish, channel catfish, leopard frog, and Fowler's toad.

Comparing these results to findings reported in the literature, as reviewed in the Introduction above (p. 1), it would appear that boron compounds are much more toxic to developmental and early posthatched stages than to adult fish. For example, Turnbull, et al. (12) gave a 24-hr TL_m of 15,000 ppm boron trifluoride for the bluegill, and Wallen, et al. (11), working with Gambusia, reported 48-hr and 96-hr LC_{50} values of 8200 and 3600 ppm borax and 10,500 and 5600 ppm boric acid. Though animal species and test conditions vary, values reported by Wallen, et al. are considerably above those found for goldfish, catfish and amphibian embryos, where LC_{50} values reported at hatching were based on exposure periods of 3-5 days (Table 25). While boron LC_1 values for piscine embryos range down to 0.001 ppm, an exposure level of 10 ppm borax is required to produce a chronic, irritant response in adult fish (36). Also, boron induces detectable frequencies of embryonic mortality and teratogenesis at concentrations significantly below those at which certain, more toxic elements (e.g., As, Hg, Pb, Zn) initiate avoidance and other behavioral responses in adult fish (37-39).

Such considerations emphasize the need to evaluate tolerances of sensitive life cycle stages in establishing protective guidelines for aquatic ecosystems. Depending on water hardness and the particular compound, boron levels of 0.001-0.1 ppm could reduce reproductive potential of more sensitive piscine species (e.g., trout), and concentrations exceeding 0.2 ppm likely would impair survival of developmental stages for other species, at least under conditons providing continuous exposure from fertilization through 4 days posthatching. As noted in the Introduction (p. 1), boron concentrations in this range are not uncommon

in U.S. freshwater resources (1-6). As water hardness, pH, total alkalinity, conductivity, and other physical and chemical parameters most likely to affect boron toxicity were well within ranges found for the majority of U.S. freshwaters (pp. 5,6; Table 4), we would expect these results to apply to most freshwater ecosystems. As maintained in these experiments, the exposure period varied from 7 days for goldfish to 28 days for trout, and developmental impairments may prove more extreme with longer exposure.

Comparative sensitivity of embryonic and posthatched stages. Results given at 4 days posthatching included a summation of 3 independent responses, embryonic mortality, teratogenesis and posthatched mortality. All three effects were observed at substantial frequencies at high boron concentrations (e.g., 50-300 ppm). However, embryonic mortality and teratogenesis were the predominant responses at lower exposure levels. Accordingly, results clearly indicated that fish and amphibian embryos are considerably more sensitive to boron compounds than early posthatched stages, at least under conditions where exposure is continuous from fertilization through 4 days posthatching. This trend is obvious from data given in Table 30, where mortality and teratogenesis were expressed per day of boron treatment. Frequencies of test responses per day were higher for embryonic stages in all but one of the 16 bioassays. The sole exception occurred when trout stages were treated with borax in soft water. Averaging results for all aquatic species, test responses per day of boron exposure were significantly greater for embryonic stages ($p < 0.001$).

Comparative toxicity of borax and boric acid. Basing exposure levels on actual boron concentration, no substantial consistent difference was observed in the order of toxicity of the two boron compounds. Combining data for all aquatic species, borax and boric acid toxicity could not be shown to differ statistically at 4 days posthatching for either water hardness level. However, certain consistent interactions were observed. Borax always was more toxic to amphibian embryos and larvae at both LC_1 and LC_{50} exposure levels. Piscine developmental stages were more sensitive to boric acid at LC_1 concentrations, due to higher frequencies of teratogenesis. This was particularly true for bioassays using hard water, where analysis of variance showed boric acid to be significantly more toxic than borax to fish embryos ($p < 0.025$). The only exception was for trout bioassays conducted in soft water, where the two compounds produced similar effects.

At intermediate and higher boron exposure levels, the catfish remained more sensitive to boric acid when administered in hard water, but no significant difference was observed between the two compounds when they were used in soft water. This is reflected in the LC_{50} values given in Table 27. However, trout and goldfish embryos and posthatched stages, like amphibians, generally were more sensitive to borax than boric acid at higher exposure levels. The only exception was for posthatched goldfish LC_{50} values determined in soft water. Combining data for all aquatic species except the catfish, borax proved more toxic at the LC_{50} level in both hard ($p < 0.02$) and soft water ($p < 0.001$). This is consistent with bioassays on adult fish reported by Wallen, et al. (11), in which borax was somewhat more toxic than boric acid to adult fish.

Effects of water hardness on boron toxicity. When LC_1 and LC_{50} data were averaged for all species, hardness level did not exert a statistically significant effect on toxicity of either borax or boric acid, though certain trends were discernible. Boron toxicity to embryonic stages generally was greater in hard water, especially for boric acid. This was most pronounced at low exposure levels, and is reflected in the LC_1 values (Table 24). Though less evident, particularly for borax, this effect also occurred in most bioassays at the LC_{50} level.

Though boron-induced embryonic mortality and teratogenesis were generally favored by hard water, there was some indication that posthatched stages were more susceptible to boron administered at the 50 ppm hardness level. This effect was observed for trout, goldfish, and leopard frog stages treated with boric acid, where percent normal survival declined more sharply from hatching to 4 days posthatching in soft water (Tables 13, 14, and 25). However, water hardness did not appear to constitute a significant variable when posthatched stages were treated with borax. The fact that water hardness does not appreciably affect boron toxicity to early fry is consistent with findings by LeClerc and Devlaminck (40) who reported 6-hr minimum lethal dosages of boric acid for adult minnows at 18,000-19,000 mg/l in distilled water and 19,000-19,500 mg/l in hard water.

Effects of other test parameters. Though efforts were not undertaken to evaluate the effects of temperature, dissolved oxygen and pH on boron toxicity, certain observations are noteworthy. In the 18 aquatic bioassays, temperature was varied from 13-29°C, dissolved oxygen ranged from 6.4-10.3 ppm,

and pH fluctuated from 7.5-8.5 (Table 4). Though moderate effects on boron toxicity could not have been deduced from the data, no substantial correlations could be established between test responses and the observed variations in temperature, oxygen, and pH.

Chick embryos. The approximate boron LC_1 and LC_{50} values for chick embryos were 0.01 and 1.0 ppm for boric acid, and 0.01 and 0.5 ppm for borax. The concentration ranges between LC_1 and LC_{50} values were considerably less than for fish and amphibian embryos, and toxicity was moderately greater with borax, primarily because of higher frequencies of embryonic teratogenesis (Tables 31, 32). The embryonic tolerance to boron is far below that of laying hens, which can survive a daily ration containing approximately 875 ppm boron (20). Results indicate that trace levels of boron in avian eggs may substantially reduce hatchability.

SUMMARY

Developmental stages of fish and amphibian embryos were treated from fertilization through 4 days posthatching with boric acid and borax, using a continuous flow bioassay system. Exposure levels were based on actual boron concentrations and each toxicant was administered at water hardness levels of 50 and 200 ppm $CaCO_3$. Test responses analyzed included embryonic mortality, embryonic teratogenesis, and posthatched (larval) mortality. Only gross debilitating anomalies were tabulated for frequencies of teratogenesis.

Developmental stages of the rainbow trout (Salmo gairdneri) were treated continuously for 28 days, with hatching occurring at 24 days (13-14°C). Combining all test responses, boric acid LC_1 and LC_{50} values, expressed in ppm boron at 4 days posthatching, were 0.1 and 100 in soft water, and 0.001 and 79 in hard water. With borax, these values were 0.07 and 27 in soft water, and 0.07 and 54 in hard water.

Embryonic and posthatched stages of the channel catfish (Ictalurus punctatus) were exposed continuously for 9 days, with hatching occurring on day 5 (25-29°C). The LC_1 and LC_{50} values for boric acid (ppm boron) were 0.5 and 155 in soft water, and 0.2 and 22 in hard water. By comparison, these values for borax were 5.5 and 155, and 1.7 and 71.

Embryos and early fry of the goldfish (Carassius auratus) were treated continuously for 7 days, with hatching occurring on day 3 (25-27°C). Boron LC_1 and LC_{50} values for boric acid in ppm were 0.6 and 46 in soft water, and

0.2 and 75 in hard water. For borax, they were 1.4 and 65 in soft water, and 0.9 and 59 in hard water.

Developmental stages of the leopard frog (Rana pipiens) received continuous treatment for 7.5 days, with hatching occurring at 3.5 days (24-25°C). Post-hatched LC_1 and LC_{50} values for boric acid were 13 and 130 ppm boron in soft water, and 22 and 135 ppm in hard water. In bioassays with borax, these values were 5 and 47 ppm, and 3 and 54 ppm.

Embryos and larvae of Fowler's toad (Bufo fowleri) were given continuous exposure for 7.5 days, with hatching occurring by 3.5 days (24-25°C). The LC_1 and LC_{50} values for boric acid were 25 and 145 ppm boron in soft water, and 5 and 123 ppm in hard water, taken at 4 days posthatching.

Generally, boron concentrations of 100-300 ppm produced complete lethality for all species, and differences in sensitivity were observed primarily at the LC_1 end of the exposure range, which extended down to 0.001 ppm boron for trout stages. Though marked differences between boric acid and borax toxicity or soft and hard water were not observed, certain distinct trends were evident. Boric acid usually was more toxic at LC_1 concentrations, and borax normally was slightly to moderately more toxic at intermediate (LC_{50}) and high boron concentrations. Though embryonic mortality and teratogenesis were greater in hard water, mortality of early fish fry and amphibian larvae was higher for most species in soft water. The latter was most evident at exposure levels of 50 ppm boron or more. While all three test responses were appreciable at higher exposure levels, embryonic mortality and teratogenesis were predominant at lower boron concentrations.

In the bioassays conducted with the 5 aquatic species, temperature ranged from 13-29°C, dissolved oxygen varied from 6.4-10.3 ppm, and pH fluctuated from 7.5-8.5. No substantial correlations were observed between these parameters and boron toxicity. Other characteristics of the prepared culture water, which was formulated to approximate the ionic composition of Mount's spring water (23), included mean values of 328 mg/l total dissolved solids, total alkalinity of 82 mg/l $CaCO_3$, conductivity of 300 umhos/cm, and an osmolarity of 12 mOsm/Kg. The total chloride content was 98 mg/l and sodium plus potassium was 30 mg/l. As the principal chemical and physical parameters most likely to affect boron toxicity were within ranges characteristic of U.S. freshwaters, test results should be applicable to most freshwater ecosystems.

Results clearly show boron compounds to be substantially more toxic to embryos and larvae than to adult fish and amphibians, emphasizing the need to evaluate tolerances of sensitive developmental stages in establishing protective guidelines for aquatic ecosystems. Depending on water hardness and the particular compound, boron levels of 0.001 to 0.1 ppm could reduce reproductive potential of more sensitive piscine species (e.g., trout), and concentrations exceeding 0.2 ppm likely would impair survival of developmental stages for other fish species, at least under conditions providing continuous exposure from fertilization through 4 days posthatching.

Tolerances for amphibian stages were higher than for fish, with 3.0 ppm boron representing the limiting LC_1 value (borax, hard water) for an exposure period of 7.5 days.

Chick embryos also were found to be much more sensitive than adult fowl to boron compounds, with an LC_1 value of 0.01 ppm boron for both boric acid and borax, and LC_{50} values of 1.0 ppm and 0.5 ppm for boric acid and borax, respectively. Treatment of chick embryos was initiated by egg yolk injection just prior to the onset of incubation, and exposure values were calculated to give ppm boron for a mean egg yolk mass of 18.4 ± 0.1 gm.

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TABLE 2

PERFORMANCE OF THE CONTINUOUS FLOW SYSTEM

Reproducibility of 1:1000 Mixing Ratio for an Aqueous Solution of Methylene Blue Dye (1000 µg/ml) Delivered at 0.3 ml/hr by a Sage Syringe Pump to Culture Chambers Receiving 300 ml/hr of Culture Water Provided by Gilson Peristaltic Pump[1]

CULTURE CHAMBER NO.	ACTUAL DYE CONCENTRATION[2,3,4] (µg/ml)
1	1.00 ±0.03
2	0.98 ±0.03
3	0.99 ±0.03
4	1.00 ±0.01
5	0.99 ±0.03
6	0.96 ±0.02

[1]The pumping rates stated above were set to give a final, theoretical dye concentration of 1 µg/ml culture water. Dye concentrations were determined spectrophotometrically (Cary model 17 recording spectrophotometer).

[2]Each concentration given as the mean value ± standard error for 20 measurements taken at regular intervals over a seven day period of continuous operation.

[3]A single Sage syringe pump (model 355) was used to operate all six syringes, and one multichannel Gilson peristaltic pump (model HP8) provided 6 separate supply lines for culture water.

[4]Sections of tygon tubing used for the different peristaltic pump channels were selected for matching diameters. Six double ground, 10 cc syringes were mounted on the syringe pump, using a modified syringe holder.

TABLE 3A. ANALYSIS OF FLOW RATES FOR BRINKMANN PERISTALTIC PUMP (MODEL 131900)[1]

Flow Rate (ml/hr)	Channel Number[2] 1	2	3	4	5	6
50	49.8±0.3	49.5±0.2	49.2±0.1	50.0±0.6	50.8±0.2	50.1±0.2
100	99.4±0.4	97.4±0.5	98.6±0.3	101.5±0.4	101.6±0.4	99.7±0.3
150	150.8±2.4	146.6±1.0	150.2±0.8	150.9±0.9	149.1±1.2	151.4±1.2
300	299.9±2.0	294.6±2.7	296.1±2.0	304.6±2.0	302.2±2.1	297.7±2.3

1 Tygon tubing - 1/16" ID, 1/8" OD.

2 Mean ± standard error; each value based on 20 replicates over a 7 day period.

TABLE 3B. ANALYSIS OF FLOW RATES FOR GILSON PERISTALTIC PUMP (MODEL HP8)[1]

Flow Rate (ml/hr)	Channel Number[2] 1	2	3	4	5	6
50	48.7±0.4	50.4±0.7	50.8±0.6	48.7±0.4	48.3±0.0	48.3±0.0
100	96.7±0.4	100.5±0.5	99.9±0.5	100.1±0.5	96.5±0.4	96.5±0.4
175	170.4±0.6	173.6±0.6	177.2±0.5	173.8±0.6	172.4±0.4	169.4±0.5
275	267.3±0.8	273.9±2.0	278.5±0.9	274.1±0.6	268.5±0.7	265.5±0.7

1 Tygon tubing - 1/16" ID, 1/8" OD.

2 Mean ± standard error; each value based on 20 replicates over a 7 day period.

TABLE 3C. ANALYSIS OF DELIVERY RATES FOR SAGE SYRINGE PUMP (MODEL 355)

Flow Rate (ml/hr X 100)	Syringe Number[1] 1	2	3	4	5	6
10.00[2]	10.07±0.24	9.80±0.17	10.00±0.16	10.21±0.21	9.53±0.13	10.42±0.1
30.00[2]	31.40±0.46	31.80±0.48	32.00±0.37	31.50±0.48	31.50±0.50	32.60±0.3
30.00[3]	30.14±0.57	30.09±0.67	32.05±0.76	31.13±0.82	30.50±0.58	30.14±0.6
60.00[3]	64.00±1.24	57.11±2.20	60.38±1.31	63.79±1.24	61.67±2.16	64.00±1.1

1 Mean ± standard error; each value based on 20 replicates over a 7 day period.

2 10 ml glass syringe (single ground).

3 10 ml plastic disposable syringe.

TABLE 4. TEST PARAMETERS OBSERVED DURING BIOASSAYS WITH BORON COMPOUNDS

BIOASSAYS (EMBRYONIC & POSTEMBRYONIC STAGES)		OBSERVED TEST PARAMETERS (MEAN ± STANDARD ERROR)				
TEST SPECIES	COMPOUND/WATER HARDNESS	TEMPERATURE (°C)	DISSOLVED OXYGEN (ppm)	WATER HARDNESS (ppm $CaCO_3$)	pH	FLOW RATE (ml/hr)
CATFISH	BORIC ACID @					
	50 ppm $CaCO_3$	25.0 ± 0.4	7.3 ± 0.1	51.8 ± 2.0	7.5 ± 0.1	194.2 ± 4.3
	200 ppm $CaCO_3$	24.7 ± 0.6	7.6 ± 0.1	212.0 ± 11.0	7.6 ± 0.0	199.7 ± 2.7
	BORAX @					
	50 ppm $CaCO_3$	29.4 ± 0.3	6.4 ± 0.1	46.7 ± 2.3	8.5 ± 0.0	188.8 ± 4.1
	200 ppm $CaCO_3$	29.4 ± 0.3	6.5 ± 0.1	194.7 ± 3.2	8.2 ± 0.1	191.0 ± 1.8
FOWLER'S TOAD	BORIC ACID @					
	50 ppm $CaCO_3$	23.7 ± 0.6	6.8 ± 0.1	57.4 ± 4.2	7.6 ± 0.1	185.3 ± 2.8
	200 ppm $CaCO_3$	23.7 ± 0.6	6.8 ± 0.1	207.5 ± 10.0	7.6 ± 0.1	189.8 ± 4.6
GOLDFISH	BORIC ACID @					
	50 ppm $CaCO_3$	24.8 ± 0.3	7.4 ± 0.0	54.4 ± 4.2	7.9 ± 0.1	184.6 ± 5.7
	200 ppm $CaCO_3$	24.8 ± 0.3	7.5 ± 0.0	207.5 ± 10.0	7.6 ± 0.1	180.4 ± 5.4
	BORAX @					
	50 ppm $CaCO_3$	27.0 ± 0.4	7.5 ± 0.2	46.2 ± 1.8	8.3 ± 0.1	208.0 ± 4.5
	200 ppm $CaCO_3$	27.0 ± 0.4	7.5 ± 0.2	194.8 ± 16.0	8.1 ± 0.1	224.2 ± 3.0
LEOPARD FROG	BORIC ACID @					
	50 ppm $CaCO_3$	25.0 ± 0.3	7.7 ± 0.2	52.5 ± 1.4	7.7 ± 0.0	193.4 ± 2.3
	200 ppm $CaCO_3$	25.0 ± 0.3	7.8 ± 0.2	212.3 ± 5.4	7.7 ± 0.0	189.2 ± 4.4
	BORAX @					
	50 ppm $CaCO_3$	25.3 ± 0.2	7.7 ± 0.2	46.2 ± 4.9	8.3 ± 0.1	180.0 ± 4.1
	200 ppm $CaCO_3$	25.3 ± 0.2	7.8 ± 0.2	203.0 ± 2.3	8.4 ± 0.1	195.6 ± 4.1
TROUT	BORIC ACID @					
	50 ppm $CaCO_3$	13.7 ± 0.1	9.2 ± 0.1	54.1 ± 3.5	7.7 ± 0.1	195.0 ± 1.2
	200 ppm $CaCO_3$	13.3 ± 0.1	9.6 ± 0.1	204.0 ± 4.0	7.9 ± 0.1	189.4 ± 2.1
	BORAX @					
	50 ppm $CaCO_3$	14.0 ± 0.1	10.1 ± 0.2	49.0 ± 1.4	7.9 ± 0.1	190.4 ± 2.9
	200 ppm $CaCO_3$	13.0 ± 0.1	10.3 ± 0.2	191.0 ± 4.0	7.8 ± 0.1	189.4 ± 2.1

TABLE 5

EXPOSURE OF TROUT EMBRYOS AND ALEVINS (SALMO GAIRDNERI)

TO BORON (BORIC ACID) IN WATER OF 50 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH)				
		% HATCHABILITY[2]	% NORMAL SURVIVAL[3]		% DEAD OR TERATOGENIC	
			H	PH	H	PH
0.001	- -	100(2)	98	96	2	4
0.01	- -	95(5)	90	89	10	11
0.10	0.11 ± 0.01	97(1)	96	96	4	4
1.00	1.00 ± 0.03	97(21)	77	77	23	23
5.00	4.74 ± 0.07	94(4)	90	84	10	16
10.00	9.26 ± 0.17	97(0)	97	97	3	3
25.00	23.50 ± 0.67	94(1)	93	93	7	7
50.00	45.50 ± 1.11	77(1)	76	76	24	24
100.00	94.00 ± 2.83	64(5)	61	58	39	42
200.00	190.00 ± 3.34	55(27)	40	27	60	73

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 6

EXPOSURE OF TROUT EMBRYOS AND ALEVINS (*SALMO GAIRDNERI*)

TO BORON (BORIC ACID) IN WATER OF 200 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	CONCENTRATION BORON (PPM) ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH)				
		% HATCHABILITY[2]	% NORMAL SURVIVAL[3]		% DEAD OR TERATOGENIC	
			H	PH	H	PH
0.001	- -	100(5)	95	93	5	7
0.01	- -	92(11)	82	79	18	21
0.10	0.10 ± 0.00	100(9)	91	89	9	11
0.50	0.47 ± 0.03	93(11)	83	81	17	19
1.00	0.98 ± 0.01	94(26)	70	68	30	32
5.00	4.85 ± 0.07	84(24)	64	63	36	37
10.00	9.40 ± 0.30	78(14)	67	67	33	33
25.00	23.80 ± 0.75	69(32)	47	46	53	54
50.00	48.30 ± 2.72	82(22)	64	63	36	37
100.00	100.20 ± 2.80	67(51)	33	33	67	67
200.00	186.00 ± 3.00	65(22)	51	44	49	56

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 7

EXPOSURE OF TROUT EMBRYOS AND ALEVINS (SALMO GAIRDNERI)

TO BORON (BORAX) IN WATER OF 50 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH) % HATCHABILITY[2]	% NORMAL SURVIVAL[3] H	% NORMAL SURVIVAL[3] PH	% DEAD OR TERATOGENIC H	% DEAD OR TERATOGENIC PH
0.001	- -	100(3)	97	97	3	3
0.01	- -	100(2)	98	98	2	2
0.10	0.12 ± 0.01	100(2)	98	98	2	2
0.50	0.49 ± 0.01	100(2)	98	98	2	2
1.00	0.96 ± 0.01	99(2)	97	96	3	4
10.00	9.70 ± 0.13	95(2)	93	86	7	14
25.00	22.60 ± 0.30	68(15)	58	57	42	43
50.00	50.60 ± 0.46	60(10)	54	22	46	78
100.00	97.40 ± 1.37	47(6)	44	0	56	100
200.00	190.20 ± 1.72	0	0	0	100	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 8

EXPOSURE OF TROUT EMBRYOS AND ALEVINS (*SALMO GAIRDNERI*)

TO BORON (BORAX) IN WATER OF 200 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH)				
		% HATCHABILITY[2]	% NORMAL SURVIVAL[3]		% DEAD OR TERATOGENIC	
			H	PH	H	PH
0.001	-	100(3)	97	97	3	3
0.01	-	100(3)	97	97	3	3
0.10	0.10 ± 0.01	100(2)	98	98	2	2
0.50	0.50 ± 0.01	100(2)	98	98	2	2
1.00	0.94 ± 0.02	98(2)	96	94	4	6
5.00	4.98 ± 0.09	97(2)	95	92	5	8
10.00	9.63 ± 0.19	93(3)	90	85	10	15
50.00	49.70 ± 0.69	80(4)	77	73	23	27
100.00	100.00 ± 2.72	55(13)	48	0	52	100
200.00	191.00 ± 2.16	0	0	0	100	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 9

EXPOSURE OF CATFISH EMBRYOS AND FRY (ICTALURUS PUNCTATUS)

TO BORON (BORIC ACID) IN WATER OF 50 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	CONCENTRATION BORON (PPM) ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH)				
		% HATCHABILITY[2]	% NORMAL SURVIVAL[3]		% DEAD OR TERATOGENIC	
			H	PH	H	PH
0.01	- -	100(0)	100	100	0	0
0.05	- -	100(0)	100	100	0	0
0.10	0.11 ± 0.01	100(0)	100	100	0	0
0.50	0.49 ± 0.01	100(1)	99	99	1	1
1.00	1.01 ± 0.02	99(0)	99	95	1	5
5.00	5.42 ± 0.29	95(2)	93	85	7	15
7.50	7.43 ± 0.12	95(3)	92	80	8	20
10.00	10.00 ± 0.05	93(5)	88	75	12	25
25.00	24.90 ± 0.19	82(8)	75	61	25	39
50.00	51.40 ± 0.88	81(5)	77	75	23	25
100.00	98.30 ± 1.14	81(6)	76	67	24	33
150.00	151.00 ± 4.20	73(10)	66	56	34	44
200.00	177.00 ± 6.39	72(10)	65	0	35	100
300.00	306.41 ±10.62	0	0	0	100	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 10

EXPOSURE OF CATFISH EMBRYOS AND FRY (ICTALURUS PUNCTATUS)

TO BORON (BORIC ACID) IN WATER OF 200 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH)				
		% HATCHABILITY[2]	% NORMAL SURVIVAL[3]		% DEAD OR TERATOGENIC	
			H	PH	H	PH
0.01	- -	100(2)	98	98	2	2
0.05	- -	100(1)	99	98	1	2
0.50	0.53 ± 0.02	100(2)	98	94	2	6
0.75	0.77 ± 0.01	100(2)	98	98	2	2
1.00	0.96 ± 0.01	96(3)	93	86	7	14
2.50	2.33 ± 0.12	99(2)	97	87	3	13
5.00	4.90 ± 0.07	95(4)	91	82	9	18
7.50	7.40 ± 0.10	95(4)	91	75	9	25
10.00	9.43 ± 0.23	94(8)	87	65	13	35
25.00	25.10 ± 0.27	90(28)	65	40	35	60
50.00	48.30 ± 0.84	83(30)	58	28	42	72
75.00	77.70 ± 0.54	71(43)	40	9	60	91
150.00	140.00 ± 5.00	57(22)	44	2	56	98
300.00	302.00 ±29.00	0	0	0	100	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 11

EXPOSURE OF CATFISH EMBRYOS AND FRY (ICTALURUS PUNCTATUS)

TO BORON (BORAX) IN WATER OF 50 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH) % HATCHABILITY[2]	% NORMAL SURVIVAL[3] H	% NORMAL SURVIVAL[3] PH	% DEAD OR TERATOGENIC H	% DEAD OR TERATOGENIC PH
0.05	- -	100(0)	100	100	0	0
0.50	0.45 ± 0.02	100(0)	100	100	0	0
1.00	0.92 ± 0.03	100(0)	100	100	0	0
5.00	4.56 ± 0.13	99(1)	98	98	2	2
7.50	7.21 ± 0.25	98(1)	97	94	3	6
10.00	9.00 ± 0.40	98(2)	96	94	4	6
25.00	25.90 ± 1.11	93(5)	88	83	12	17
50.00	49.80 ± 3.74	85(7)	79	70	21	30
75.00	72.60 ± 2.01	85(6)	80	79	20	21
100.00	93.30 ± 3.72	85(22)	66	56	34	44
150.00	149.00 ± 4.01	80(19)	65	65	35	35
200.00	192.00 ± 9.30	73(10)	66	0	34	100
300.00	301.00 ±13.60	0	0	0	100	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 12

EXPOSURE OF CATFISH EMBRYOS AND FRY (ICTALURUS PUNCTATUS)

TO BORON (BORAX) IN WATER OF 200 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH) % HATCHABILITY[2]	% NORMAL SURVIVAL[3] H	% NORMAL SURVIVAL[3] PH	% DEAD OR TERATOGENIC H	% DEAD OR TERATOGENIC PH
0.05	- -	100(0)	100	100	0	0
0.10	0.10 ± 0.02	100(1)	99	99	1	1
0.50	0.49 ± 0.02	100(0)	100	100	0	0
1.00	1.04 ± 0.04	100(4)	96	96	4	4
5.00	4.60 ± 0.09	99(4)	95	94	5	6
7.50	6.23 ± 0.15	98(6)	94	94	6	6
10.00	8.82 ± 0.28	98(3)	95	90	5	10
25.00	29.50 ± 1.02	79(5)	75	71	25	29
50.00	49.10 ± 2.65	80(8)	74	61	26	39
100.00	103.00 ± 4.17	72(26)	51	38	49	62
200.00	197.00 ±12.70	52(23)	40	2	60	98
300.00	320.00 ±16.70	16(100)	0	0	100	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 13

EXPOSURE OF GOLDFISH EMBRYOS AND FRY (*CARASSIUS AURATUS*)

TO BORON (BORIC ACID) IN WATER OF 50 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH) % HATCHABILITY[2]	% NORMAL SURVIVAL[3] H	% NORMAL SURVIVAL[3] PH	% DEAD OR TERATOGENIC H	% DEAD OR TERATOGENIC PH
0.05	- -	99(0)	99	99	1	1
0.10	0.10 ± 0.01	97(1)	96	96	4	4
0.50	0.49 ± 0.04	98(0)	98	98	2	2
1.00	0.90 ± 0.04	98(0)	98	98	2	2
5.00	5.20 ± 0.48	99(1)	98	98	2	2
7.50	7.00 ± 0.10	93(0)	93	93	7	7
10.00	9.20 ± 0.23	96(2)	94	94	6	6
25.00	22.50 ± 2.50	86(1)	85	78	15	22
50.00	48.70 ± 3.70	69(7)	64	52	36	48
100.00	108.00 ±22.00	66(9)	60	4	40	96
200.00	188.70 ± 5.80	53(8)	49	0	51	100
300.00	288.00 ± 3.46	34(76)	8	0	92	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 14

EXPOSURE OF GOLDFISH EMBRYOS AND FRY (*CARASSIUS AURATUS*)

TO BORON (BORIC ACID) IN WATER OF 200 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH) % HATCHABILITY[2]	% NORMAL SURVIVAL[3] H	% NORMAL SURVIVAL[3] PH	% DEAD OR TERATOGENIC H	% DEAD OR TERATOGENIC PH
0.05	- -	98(1)	97	97	3	3
0.10	0.12 ± 0.01	98(2)	96	96	4	4
0.50	0.47 ± 0.04	95(0)	95	95	5	5
1.00	0.90 ± 0.02	98(0)	98	98	2	2
5.00	4.50 ± 0.26	97(1)	96	96	4	4
7.50	6.80 ± 0.30	94(1)	93	93	7	7
10.00	8.33 ± 0.48	85(0)	85	85	15	15
25.00	32.00 ± 4.00	79(1)	78	76	22	24
50.00	51.30 ± 4.70	80(0)	80	67	20	33
100.00	96.70 ± 1.76	61(3)	59	35	41	65
200.00	191.00 ± 2.00	56(14)	48	0	52	100
300.00	290.00 ± 6.00	35(40)	21	0	79	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 15

EXPOSURE OF GOLDFISH EMBRYOS AND FRY (CARASSIUS AURATUS)

TO BORON (BORAX) IN WATER OF 50 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH) % HATCHABILITY[2]	% NORMAL SURVIVAL[3] H	% NORMAL SURVIVAL[3] PH	% DEAD OR TERATOGENIC H	% DEAD OR TERATOGENIC PH
0.05	- -	99(1)	98	98	2	2
0.10	0.07 ± 0.01	99(0)	99	99	1	1
0.50	0.54 ± 0.04	98(0)	98	97	2	3
1.00	0.94 ± 0.05	97(0)	97	97	3	3
5.00	4.68 ± 0.13	93(0)	93	93	7	7
7.50	7.43 ± 0.44	94(1)	93	93	7	7
10.00	9.10 ± 0.33	93(1)	92	91	8	9
25.00	26.50 ± 3.05	93(1)	92	92	8	8
50.00	48.75 ± 0.95	88(7)	82	78	18	22
100.00	96.00 ± 3.92	65(40)	39	4	61	96
200.00	189.67 ± 7.06	0	0	0	100	100
300.00	327.60 ±12.67	0	0	0	100	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 16

EXPOSURE OF GOLDFISH EMBRYOS AND FRY (CARASSIUS AURATUS)

TO BORON (BORAX) IN WATER OF 200 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH)				
		% HATCHABILITY[2]	% NORMAL SURVIVAL[3]		% DEAD OR TERATOGENIC	
			H	PH	H	PH
0.05	- -	99(0)	99	99	1	1
0.10	0.08 ± 0.03	98(2)	96	96	4	4
0.50	0.69 ± 0.10	98(0)	98	98	2	2
1.00	0.89 ± 0.05	95(0)	95	95	5	5
5.00	4.10 ± 0.15	95(3)	92	92	8	8
7.50	6.47 ± 0.09	94(2)	92	92	8	8
10.00	8.53 ± 0.29	94(1)	93	93	7	7
25.00	27.33 ± 3.00	91(4)	87	85	13	15
50.00	42.25 ± 4.03	89(6)	84	77	16	23
100.00	88.67 ± 1.76	65(45)	36	0	64	100
200.00	190.00 ± 2.31	0	0	0	100	100
300.00	303.00 ± 9.00	0	0	0	100	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 17

EXPOSURE OF LEOPARD FROG EMBRYOS AND LARVAE (*RANA PIPIENS*)

TO BORON (BORIC ACID) IN WATER OF 50 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	CONCENTRATION BORON (PPM) ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH)				
		% HATCHABILITY[2]	% NORMAL SURVIVAL[3]		% DEAD OR TERATOGENIC	
			H	PH	H	PH
0.05	- -	99(0)	99	96	1	4
0.10	0.09 ± 0.01	100(1)	99	99	1	1
0.50	0.39 ± 0.03	100(0)	100	99	0	1
1.00	0.93 ± 0.01	100(1)	99	99	1	1
5.00	4.50 ± 0.10	98(1)	97	97	3	3
10.00	9.15 ± 0.29	99(1)	98	93	2	7
25.00	32.50 ± 4.50	99(4)	95	91	5	9
50.00	47.50 ± 2.18	98(2)	96	91	4	9
100.00	84.50 ± 2.87	96(3)	93	87	7	13
200.00	188.00 ± 6.93	90(60)	36	16	64	84
300.00	298.00 ±16.40	77(100)	0	0	100	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 18

EXPOSURE OF LEOPARD FROG EMBRYOS AND LARVAE (*RANA PIPIENS*)

TO BORON (BORIC ACID) IN WATER OF 200 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH) % HATCHABILITY[2]	% NORMAL SURVIVAL[3] H	% NORMAL SURVIVAL[3] PH	% DEAD OR TERATOGENIC H	% DEAD OR TERATOGENIC PH
0.10	0.13 ± 0.01	100(1)	99	99	1	1
0.50	0.53 ± 0.09	100(1)	99	99	1	1
1.00	0.92 ± 0.02	100(0)	100	100	0	0
5.00	4.60 ± 0.15	97(1)	96	94	4	6
10.00	8.93 ± 0.27	98(1)	97	97	3	3
25.00	25.20 ± 0.44	96(0)	96	95	4	5
50.00	45.70 ± 1.67	96(2)	94	94	6	6
100.00	86.00 ± 3.46	94(2)	92	88	8	12
200.00	193.00 ± 8.11	87(68)	29	14	71	86
300.00	288.00 ±16.00	49(100)	0	0	100	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 19

EXPOSURE OF LEOPARD FROG EMBRYOS AND LARVAE (*RANA PIPIENS*)

TO BORON (BORAX) IN WATER OF 50 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH)				
		% HATCHABILITY[2]	% NORMAL SURVIVAL[3]		% DEAD OR TERATOGENIC	
			H	PH	H	PH
0.05	- -	100(1)	99	98	1	2
0.10	0.11 ± 0.01	98(1)	97	96	3	4
0.50	0.51 ± 0.03	100(1)	99	99	1	1
1.00	0.84 ± 0.07	100(1)	99	99	1	1
5.00	4.57 ± 0.09	99(1)	98	96	2	4
7.50	7.04 ± 0.28	99(2)	97	96	3	4
10.00	9.60 ± 0.28	95(1)	94	93	6	7
25.00	27.30 ± 2.25	92(6)	86	84	14	16
50.00	56.00 ± 6.00	75(31)	52	39	48	61
100.00	103.00 ±10.30	25(88)	3	2	97	98
200.00	205.00 ± 5.00	22(100)	0	0	100	100
300.00	292.00 ±10.00	14(100)	0	0	100	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 20

EXPOSURE OF LEOPARD FROG EMBRYOS AND LARVAE (RANA PIPIENS)

TO BORON (BORAX) IN WATER OF 200 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH) % HATCHABILITY[2]	% NORMAL SURVIVAL[3] H	% NORMAL SURVIVAL[3] PH	% DEAD OR TERATOGENIC H	% DEAD OR TERATOGENIC PH
0.05	- -	100(1)	99	99	1	1
0.10	0.13 ± 0.01	99(2)	97	97	3	3
0.50	0.42 ± 0.04	99(1)	98	98	2	2
1.00	1.00 ± 0.04	100(1)	99	99	1	1
5.00	4.98 ± 0.31	95(2)	93	92	7	8
7.50	7.04 ± 0.28	94(1)	93	91	7	9
10.00	10.50 ± 1.04	93(2)	91	90	9	10
25.00	28.40 ± 1.78	88(10)	79	76	21	24
50.00	55.20 ± 3.81	75(21)	59	52	41	48
100.00	108.00 ±11.30	52(35)	26	16	74	84
200.00	244.00 ±15.30	18(100)	0	0	100	100
300.00	328.00 ± 9.25	4(100)	0	0	100	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 21

EXPOSURE OF FOWLER'S TOAD EMBRYOS AND LARVAE (BUFO FOWLERI)

TO BORON (BORIC ACID) IN WATER OF 50 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH)				
		% HATCHABILITY[2]	% NORMAL SURVIVAL[3]		% DEAD OR TERATOGENIC	
			H	PH	H	PH
0.05	- -	100(1)	99	99	1	1
0.10	0.10 ± 0.01	100(2)	98	98	2	2
0.50	0.46 ± 0.14	100(1)	99	99	1	1
1.00	0.97 ± 0.12	100(3)	97	97	3	3
5.00	5.00 ± 0.38	100(2)	98	98	2	2
10.00	9.40 ± 0.40	100(1)	99	99	1	1
25.00	24.00 ± 2.10	100(2)	98	98	2	2
50.00	48.70 ± 3.70	100(1)	99	98	1	2
100.00	96.00 ±17.50	98(24)	74	73	26	27
200.00	189.00 ± 6.90	91(96)	4	0	96	100
300.00	279.00 ± 9.00	81(100)	0	0	100	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 22

EXPOSURE OF FOWLER'S TOAD EMBRYOS AND LARVAE (BUFO FOWLERI)

TO BORON (BORIC ACID) IN WATER OF 200 PPM $CaCO_3$ HARDNESS

CONCENTRATION BORON (PPM) NOMINAL	ACTUAL[1]	TEST RESPONSES AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH) % HATCHABILITY[2]	% NORMAL SURVIVAL[3] H	% NORMAL SURVIVAL[3] PH	% DEAD OR TERATOGENIC H	% DEAD OR TERATOGENIC PH
0.05	- -	100(2)	98	98	2	2
0.10	- -	100(2)	98	98	2	2
0.50	0.45 ± 0.04	100(3)	97	97	3	3
1.00	0.93 ± 0.04	100(2)	98	98	2	2
5.00	4.50 ± 0.20	95(2)	93	93	7	7
10.00	8.30 ± 0.30	95(2)	93	93	7	7
25.00	22.30 ± 3.30	96(2)	94	94	6	6
50.00	53.50 ± 4.50	96(24)	73	72	27	28
100.00	93.00 ± 1.00	94(30)	66	65	34	35
200.00	204.00 ± 4.00	91(90)	9	0	91	100
300.00	308.00 ± 8.60	31(94)	2	0	98	100

[1]Concentration given as mean ± standard error as determined by curcumin method.

[2]Percent of surviving population bearing gross teratogenic defects is given parenthetically.

[3]Percent of initial population surviving with normal morphology.

TABLE 23. SURVIVAL FREQUENCIES FOR CONTROL POPULATIONS OF AQUATIC EMBRYOS

EMBRYONIC BIOASSAYS		COMBINED POPULATION SIZE	% CONTROL SURVIVAL[1]	
TEST SPECIES	COMPOUND/WATER HARDNESS		AT HATCHING	AT 4 DAYS POSTHATCHING
CATFISH	BORIC ACID @			
	50 ppm $CaCO_3$	304	93	91
	200 ppm $CaCO_3$	258	94	93
	BORAX @			
	50 ppm $CaCO_3$	371	97	97
	200 ppm $CaCO_3$	368	98	98
FOWLER'S TOAD	BORIC ACID @			
	50 ppm $CaCO_3$	443	92	92
	200 ppm $CaCO_3$	410	94	94
GOLDFISH	BORIC ACID @			
	50 ppm $CaCO_3$	249	94	92
	200 ppm $CaCO_3$	294	93	92
	BORAX @			
	50 ppm $CaCO_3$	249	94	92
	200 ppm $CaCO_3$	294	93	92
LEOPARD FROG	BORIC ACID @			
	50 ppm $CaCO_3$	434	90	89
	200 ppm $CaCO_3$	404	89	87
	BORAX @			
	50 ppm $CaCO_3$	434	90	89
	200 ppm $CaCO_3$	404	89	87
TROUT	BORIC ACID @			
	50 ppm $CaCO_3$	242	91	91
	200 ppm $CaCO_3$	252	92	87
	BORAX @			
	50 ppm $CaCO_3$	242	91	91
	200 ppm $CaCO_3$	252	92	87

[1]The mean frequency of teratogenic animals appearing in control populations was 0.6%.

TABLE 24

LOG PROBIT ANALYSIS OF LC_1 VALUES IN PPM

FOR AQUATIC EMBRYOS EXPOSED TO BORON COMPOUNDS[1]

TEST SPECIES	BORIC ACID				BORAX			
	50 ppm $CaCO_3$		200 ppm $CaCO_3$		50 ppm $CaCO_3$		200 ppm $CaCO_3$	
	H	PH	H	PH	H	PH	H	PH
CATFISH	1.0	0.5	0.3	0.2	5.5	5.5	1.7	1.7
FOWLER'S TOAD	25.0	25.0	5.0	5.0	-	-	-	-
GOLDFISH	0.6	0.6	0.2	0.2	1.4	1.4	0.9	0.9
LEOPARD FROG	26.0	13.0	23.0	22.0	6.0	5.0	3.0	3.0
TROUT	0.1	0.1	0.001	0.001	0.07	0.07	0.07	0.07

[1] LC_1 values were calculated at hatching (H) and 4 days posthatching (PH) for two water hardness levels (50 ppm $CaCO_3$, 200 ppm $CaCO_3$). Grossly anomalous survivors were counted as lethals.

TABLE 25

LOG PROBIT ANALYSIS OF LC_{50} VALUES IN PPM

FOR AQUATIC EMBRYOS EXPOSED TO BORON COMPOUNDS[1]

TEST SPECIES	BORIC ACID				BORAX			
	50 ppm $CaCO_3$		200 ppm $CaCO_3$		50 ppm $CaCO_3$		200 ppm $CaCO_3$	
	H	PH	H	PH	H	PH	H	PH
CATFISH	220*	155*	102	22	235*	155*	120*	71*
FOWLER'S TOAD	148	145	135	123	-	-	-	-
GOLDFISH	178*	46*	170	75*	71	65*	68	59*
LEOPARD FROG	157	130*	145	135*	54*	47*	60*	54*
TROUT	150	100	100	79*	65*	27*	88	54*

[1]Values were calculated at hatching (H) and 4 days posthatching (PH) for two water hardness levels (50 ppm $CaCO_3$, 200 ppm $CaCO_3$). Grossly anomalous survivors were counted as lethals. Asterisks indicate values determined by the Litchfield-Wilcoxon graphic method.

TABLE 26

95% CONFIDENCE LIMITS FOR LC_1 VALUES IN PPM FOR AQUATIC EMBRYOS EXPOSED TO BORON COMPOUNDS[1]

COMPOUND/ SPECIES	EXPOSURE TIME	50 ppm $CaCO_3$			200 ppm $CaCO_3$		
		LC_1	CONFIDENCE LIMITS LOWER	UPPER	LC_1	CONFIDENCE LIMITS LOWER	UPPER
BORIC ACID							
CATFISH	H	1.00	0.02	4.40	0.30	0.00	2.30
	PH	0.50	0.01	2.10	0.20	0.00	1.80
FOWLER'S TOAD	H	25.00	-	-	5.00	-	-
	PH	25.00	-	-	5.00	-	-
GOLDFISH	H	0.60	0.00	5.40	0.20	0.01	0.90
	PH	0.60	0.00	5.40	0.20	0.01	0.90
LEOPARD FROG	H	26.00	-	-	23.00	-	-
	PH	13.00	-	-	22.00	-	-
TROUT	H	0.10	-	-	0.001	0.0001	0.006
	PH	0.10	-	-	0.001	0.0001	0.003
BORAX							
CATFISH	H	5.50	0.10	17.30	1.70	0.10	11.90
	PH	5.50	0.10	17.30	1.70	0.10	11.90
GOLDFISH	H	1.40	0.00	8.00	0.90	0.01	3.50
	PH	1.40	0.00	8.00	0.90	0.01	3.50
LEOPARD FROG	H	6.00	-	-	3.00	0.50	6.90
	PH	5.00	-	-	3.00	0.60	6.00
TROUT	H	0.07	-	-	0.07	-	-
	PH	0.07	-	-	0.07	-	-

[1] LC_1 values were calculated at hatching (H) and 4 days posthatching (PH) for two water hardness levels (50 ppm $CaCO_3$, 200 ppm $CaCO_3$). Grossly anomalous survivors were counted as lethals.

TABLE 27

95% CONFIDENCE LIMITS FOR LC_{50} VALUES IN PPM FOR AQUATIC EMBRYOS EXPOSED TO BORON COMPOUNDS[1]

COMPOUND/ SPECIES	EXPOSURE TIME	50 ppm $CaCO_3$			200 ppm $CaCO_3$		
		LC_{50}	CONFIDENCE LIMITS LOWER	UPPER	LC_{50}	CONFIDENCE LIMITS LOWER	UPPER
BORIC ACID							
CATFISH	H	220.0	167.0	290.0	102.0	23.0	180.0
	PH	155.0	111.0	217.0	22.0	19.0	25.0
FOWLER'S TOAD	H	148.0	120.0	183.0	135.0	104.0	175.0
	PH	145.0	118.0	179.0	123.0	94.0	161.0
GOLDFISH	H	178.0	131.0	242.0	170.0	115.0	251.0
	PH	46.0	32.0	66.0	75.0	50.0	112.0
LEOPARD FROG	H	157.0	127.0	194.0	145.0	117.0	180.0
	PH	130.0	99.0	171.0	135.0	109.0	168.0
TROUT	H	150.0	90.0	249.0	100.0	61.0	163.0
	PH	100.0	70.0	142.0	79.0	35.0	165.0
BORAX							
CATFISH	H	235.0	193.0	287.0	120.0	79.0	165.0
	PH	155.0	121.0	198.0	71.0	43.0	109.0
GOLDFISH	H	71.0	51.0	98.0	68.0	41.0	112.0
	PH	65.0	42.0	102.0	59.0	36.0	96.0
LEOPARD FROG	H	54.0	42.0	70.0	60.0	49.0	74.0
	PH	47.0	39.0	57.0	54.0	45.0	65.0
TROUT	H	65.0	40.0	106.0	88.0	48.0	160.0
	PH	27.0	16.0	48.0	54.0	28.0	105.0

[1]Values were calculated at hatching (H) and 4 days posthatching (PH) for two water hardness levels (50 ppm $CaCO_3$, 200 ppm $CaCO_3$). Grossly anomalous survivors were counted as lethals.

TABLE 28

SYRINGE PUMP (BORON) AND PERISTALTIC PUMP (CULTURE WATER) FLOW RATES WITH RESULTING DILUTION RATIOS OBTAINED IN TREATMENT OF TROUT EMBRYOS AT 1 AND 10 PPB BORON[1]

TROUT BIOASSAYS		SYRINGE PUMP (BORON)			PERISTALTIC PUMP (CULTURE WATER)		FINAL BORON CONCENTRATION[2] (µg/l)
COMPOUND HARDNESS	NOMINAL BORON LEVEL (µg/l)	FLOW (µl/hr)	BORON (µg/l) THEORETICAL	BORON (µg/l) ACTUAL	FLOW (ml/hr)	BORON DILUTION RATIO	
BORIC ACID							
50 ppm $CaCO_3$	1.0	330 ± 3	600	594 ± 4	198 ± 2.4	600 ± 7	0.99
	10.0	330 ± 3	6000	6050 ± 90	198 ± 4.8	600 ± 15	10.08
200 ppm $CaCO_3$	1.0	318 ± 7	600	614 ± 4	183 ± 4.0	575 ± 13	1.07
	10.0	306 ± 1	6000	6130 ± 60	192 ± 3.7	627 ± 8	9.78
BORAX							
50 ppm $CaCO_3$	1.0	318 ± 4	600	588 ± 11	188 ± 2.9	591 ± 14	0.99
	10.0	325 ± 3	6000	5930 ± 80	190 ± 2.8	585 ± 11	10.14
200 ppm $CaCO_3$	1.0	320 ± 1	600	594 ± 4	194 ± 1.9	606 ± 7	0.98
	10.0	320 ± 1	6000	5870 ± 70	197 ± 1.3	616 ± 5	9.53

[1]Mean values ± standard errors, where n was 10 for syringe pump boron analyses and 20 for all other determinations.

[2]Final boron concentration delivered to the culture chamber was determined as actual syringe pump boron concentration/boron dilution ratio. The latter was calculated as peristaltic pump flow rate/syringe pump flow rate.

TABLE 29. TERATOGENESIS IN AQUATIC EMBRYOS SURVIVING TREATMENT WITH BORIC ACID AND BORAX

BIOASSAYS			PERCENT DISTRIBUTION OF ANOMALIES BY AREAS AFFECTED					
SPECIES	TOXICANT & HARDNESS	NO. ANIMALS ANOMALOUS[1]	DWARFED BODY	CRANIUM	VERTEBRAL COLUMN	FINS	NERVOUS SYSTEM	YOLK SAC & ABDOMEN
CATFISH	BORIC ACID @							
	50 ppm $CaCO_3$	77	6	3	53	38	-	-
	200 ppm $CaCO_3$	125	2	5	93	-	-	-
	BORAX @							
	50 ppm $CaCO_3$	84	38	2	55	-	-	5
	200 ppm $CaCO_3$	107	21	2	75	-	2	-
GOLDFISH	BORIC ACID @							
	50 ppm $CaCO_3$	65	-	2	93	5	-	-
	200 ppm $CaCO_3$	26	-	-	100	-	-	-
	BORAX @							
	50 ppm $CaCO_3$	44	2	-	93	-	-	5
	200 ppm $CaCO_3$	68	-	-	91	2	-	7
TROUT	BORIC ACID @							
	50 ppm $CaCO_3$	58	2	10	84	-	4	-
	200 ppm $CaCO_3$	278	3	8	80	2	2	5
	BORAX @							
	50 ppm $CaCO_3$	40	10	25	40	3	5	17
	200 ppm $CaCO_3$	33	3	13	63	6	15	-
FOWLER'S TOAD	BORIC ACID @							
	50 ppm $CaCO_3$	810	-	1	16	-	-	83
	200 ppm $CaCO_3$	544	-	1	12	-	-	87
LEOPARD FROG	BORIC ACID @							
	50 ppm $CaCO_3$	209	-	-	6	-	-	94
	200 ppm $CaCO_3$	190	-	-	33	-	-	67
	BORAX @							
	50 ppm $CaCO_3$	155	-	1	49	-	-	50
	200 ppm $CaCO_3$	151	-	1	92	1	-	6

[1]Includes anomalous survivors for all exposure levels

TABLE 30

COMPARISON OF SENSITIVITY OF EMBRYONIC AND POSTHATCHED STAGES TO BORON COMPOUNDS

BIOASSAYS		PERCENT ANIMALS DEAD OR TERATOGENIC PER DAY BORON TREATMENT[1]			
		BORIC ACID		BORAX	
SPECIES	STAGE	50 ppm $CaCO_3$	200 ppm $CaCO_3$	50 ppm $CaCO_3$	200 ppm $CaCO_3$
Trout	Embryonic	0.8	1.3	1.1	1.7
	Posthatched	0.6	0.5	2.1	1.5
Catfish	Embryonic	3.9	4.9	4.1	4.7
	Posthatched	2.4	3.3	1.8	1.5
Goldfish	Embryonic	7.2	7.1	8.8	9.1
	Posthatched	2.7	2.2	0.8	0.9
Leopard Frog	Embryonic	4.9	5.7	8.9	8.7
	Posthatched	1.0	0.5	0.5	0.5

[1]For each experimental series, data were averaged for all boron exposure levels.

TABLE 31

TOXICITY OF BORIC ACID TO CHICK EMBRYOS

CONCENTRATION BORON (PPM)	% SURVIVAL[1]	% SURVIVORS ANOMALOUS
0.01	92	0
0.05	89	0
0.10	84	0
0.25	75	0
0.50	60	4
0.75	58	4
1.00	54	4
2.50	44	7
5.00	38	5
10.00	21	10
25.00	0	-
50.00	0	-

[1]Percent survival includes all embryos which completed hatching.

TABLE 32

TOXICITY OF BORAX TO CHICK EMBRYOS

CONCENTRATION BORON (PPM)	% SURVIVAL[1]	% SURVIVORS ANOMALOUS
0.01	95	0
0.05	84	0
0.10	73	3
0.25	64	2
0.50	58	4
0.75	51	8
1.00	49	9
2.50	42	10
5.00	35	13
10.00	25	28
25.00	11	42
50.00	0	-

[1]Percent survival includes all embryos which completed hatching.

TABLE 33

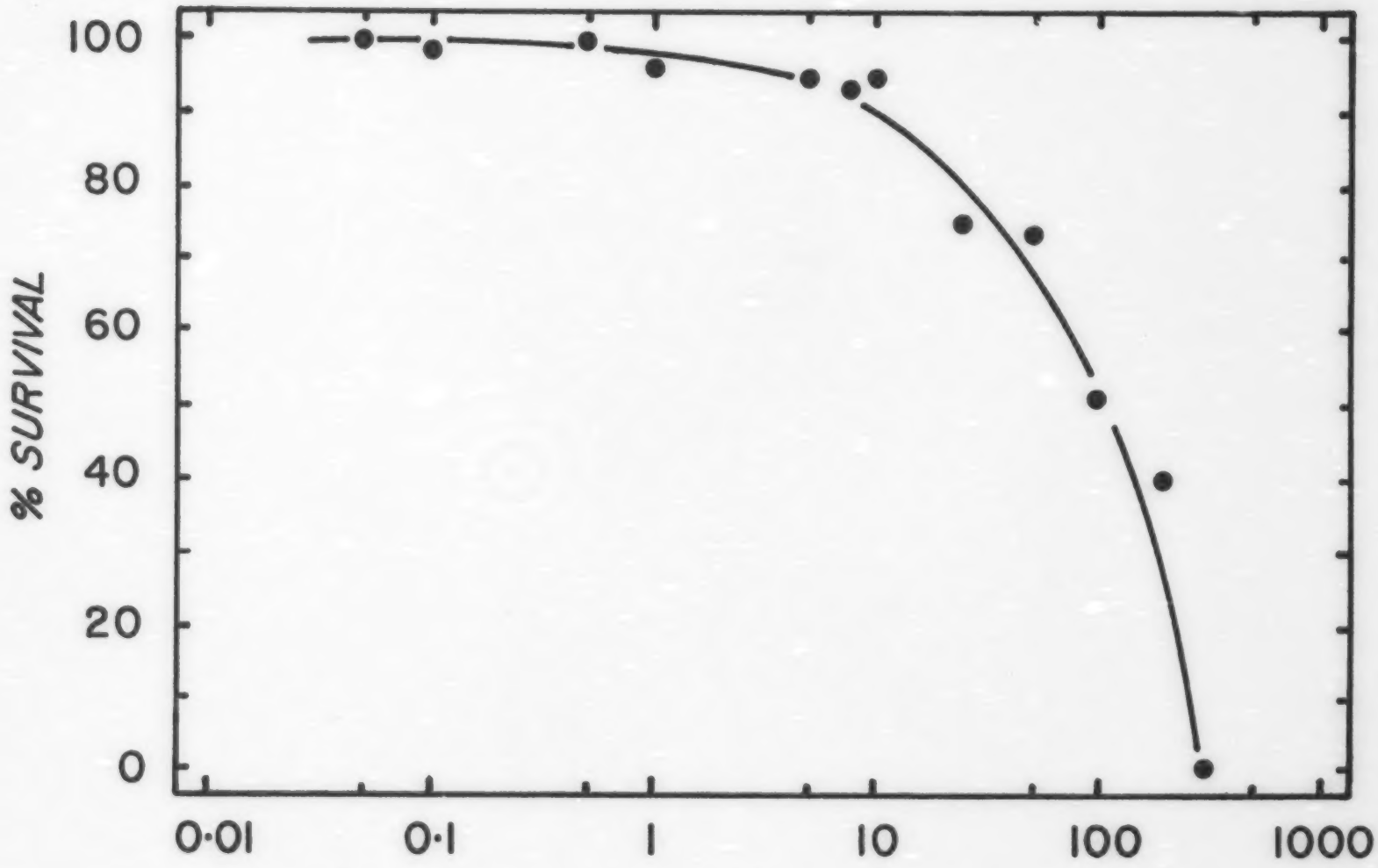
EFFECT OF FLOW RATE ON

METAL TOXICITY TO CATFISH EMBRYOS AND ALEVINS

FLOW RATE (ml/hr)	TURNOVER RATE (per hour)[1]	PERCENT SURVIVAL AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH) FOR METAL CONCENTRATIONS IN PPM								
		Hg^{+2}	H	PH	Cd^{+2}	H	PH	Zn^{+2}	H	PH
50	0.2	0.0021	36	17	0.23	40	13	0.42	57	40
125	0.5	0.0027	45	23	0.25	48	22	0.50	59	38
250	1.0	0.0025	46	25	0.29	54	32	0.50	60	34
400	1.6	0.0024	43	25	0.30	50	27	0.59	61	35

[1]Values represent portion of a 250 ml culture chamber renewed per hour.

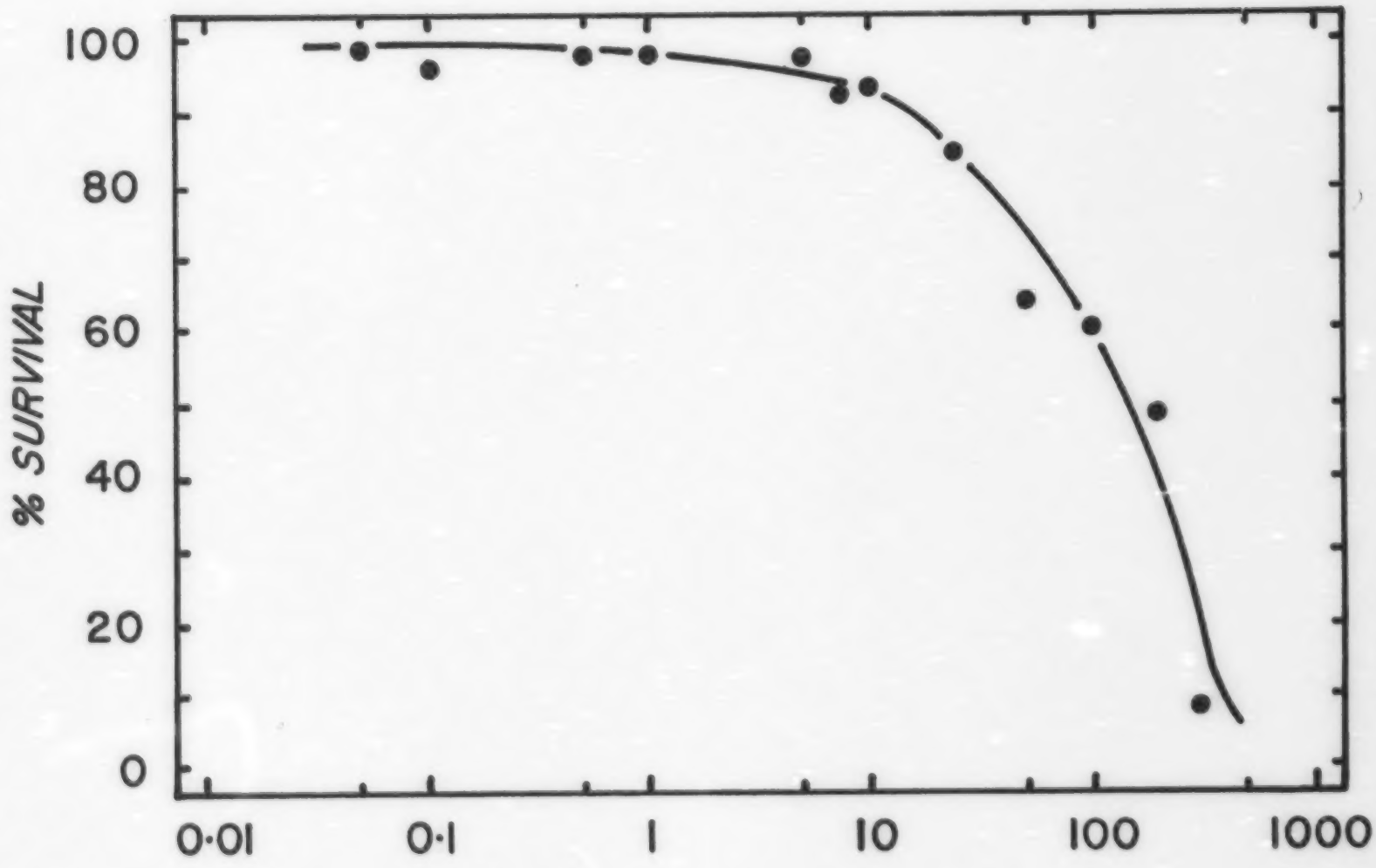
FIGURE 3



TOXICITY OF BORAX TO CATFISH EMBRYOS

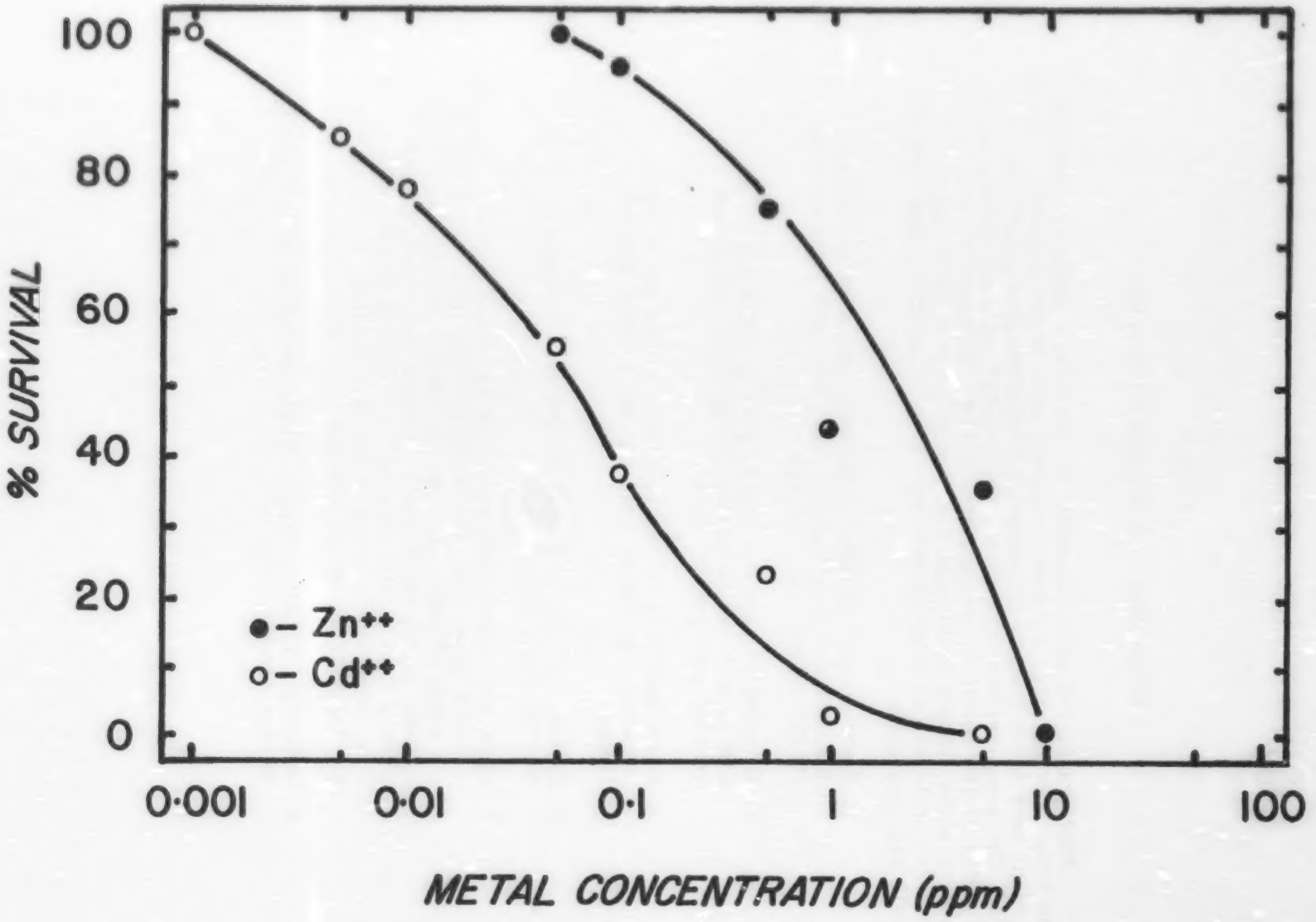
09

FIGURE 4



TOXICITY OF BORIC ACID TO GOLDFISH EMBRYOS

FIGURE 5



TOXICITY OF METALS TO GOLDFISH EMBRYOS

PLATE 1

BORON-INDUCED TERATOGENESIS IN TROUT

Photographs 1-6 show congenital deformities frequently observed in trout alevins which survived treatment with boron compounds. Photographs were taken 2 weeks subsequent to hatching at a magnification of 10X. The anomalies most frequently encountered included various acute defects of the vertebral column, amphiarthrotic (immobile) jaw articulation, absent or reduced fins, anomalous or absent eyes, dwarfed or irregular trunk morphology, Siamese twinning, and retarded yolk sac resorption.

1. Boron-induced skeletal anomalies including an acute, lordotic spine, attenuated tail fin, and a defective, immobile lower jaw (arrow).

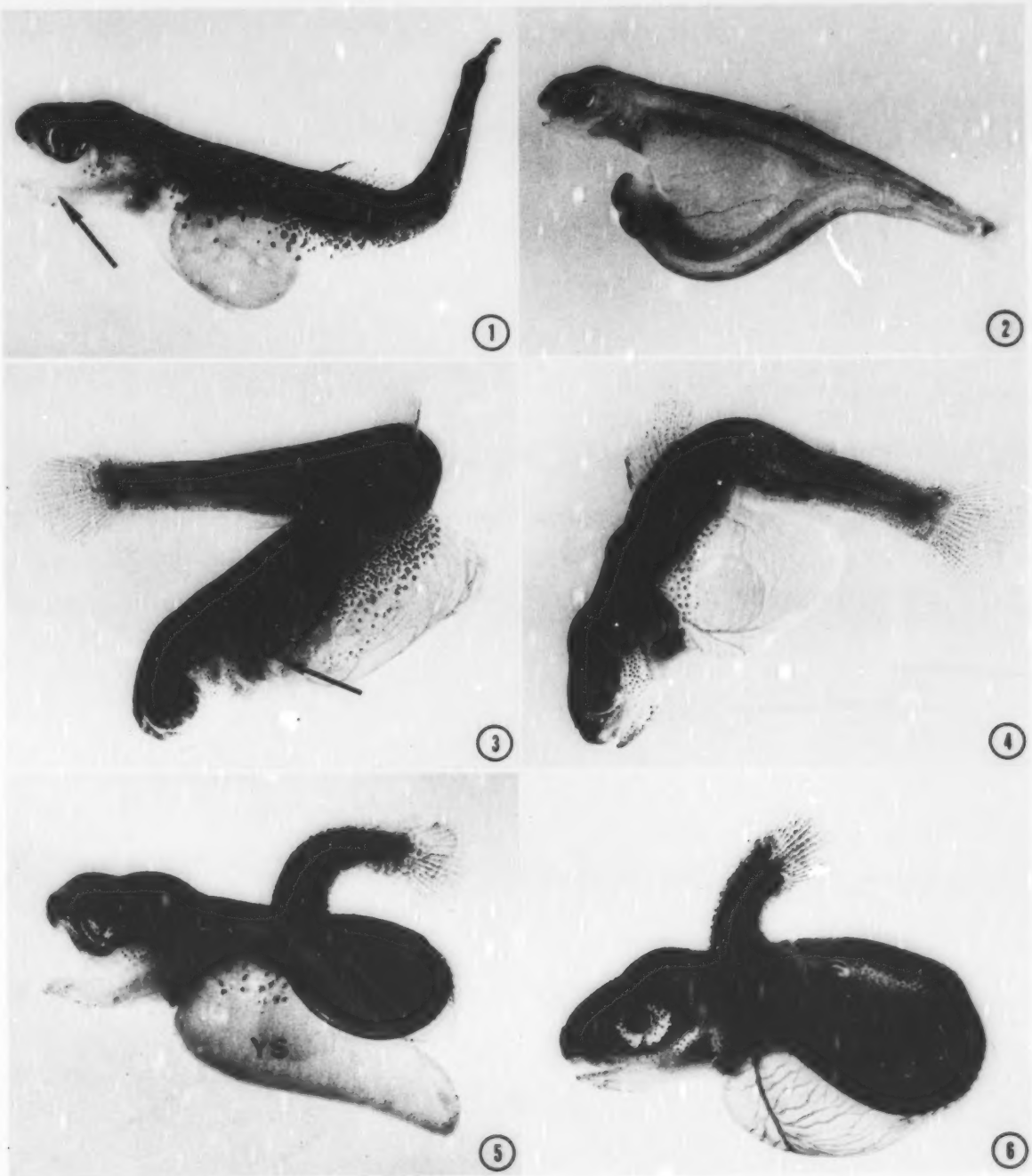
2. Twinning of head and trunk, with a single tail. A single yolk sac possesses paired yolk stalks, one serving each partial twin.

3. Acute scoliosis of the vertebral column and cephalic twinning. Secondary head (arrow) is only partially formed.

4. Kyphosis of the vertebral column with irregular, dwarfed trunk structure.

5. Anomalous, inflexible curvature of spinal column; irregular, truncate upper jaw, and immobile lower jaw. As shown in all other figures, yolk sac resorption is retarded. Also, as seen in Figures 1 and 6, incomplete closure of the choroid fissure has resulted in coloboma of the retina. This condition is discernible as a vertical cleft at the mid-inferior border of the eye.

6. Irregular, fixed curvature of the vertebral column similar to that shown in Figure 5.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